ELECTROFISHING-INDUCED INJURY TO FISH: EFFECT OF SPECIES, DEVELOPMENTAL STAGE, AND ELECTRIC-FIELD CHARACTERISTICS

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VITA

Theodore Burdick Henry, son of Arden Burdick and Mary Feltner Henry, was born on 28 May 1970, in Buffalo, New York. He graduated from Santa Barbara High School, Santa Barbara, California in 1988. He received a Bachelor of Arts in Aquatic Biology in June 1992 from the University of California Santa Barbara. In July 1992 he entered the United States Peace Corps and worked as a fisheries extension/rural community development agent in Togo, West Africa, until November 1994. In fall 1995 he entered the Department of Fisheries and Allied Aquacultures at Auburn University and began research for his Master of Science degree in the Alabama Cooperative Fish and Wildlife Research Unit. He completed his Master of Science degree, on the toxicity of acid mine drainage mixing zones to warmwater fish, in March 1998. Upon completion of his Master of Science, he obtained a research assistantship in the Department of Fisheries and Allied Aquacultures, Southeastern Cooperative Fish Disease Project, Auburn University. In November 2001, he was engaged to Katia Marioni, daughter of Maria and Gildo Marioni of Parma, Italy.

PREFACE

Electrofishing is an integral sampling tool of fisheries management programs across the U.S. However, injury and mortality to target species has caused the use of electrofishing to come under criticism in recent years by some in the scientific community and the popular press. The bulk of the published information to date reporting such injury has been focused on coldwater species, notably salmonids. The Fisheries Management Section (FMS) of the American Fisheries Society felt the need to examine potential for electrofishing injury in selected warmwater species and make recommendations on procedures to minimize potential for negative impacts to target and non-target species.

FMS procured funding from the U.S. Fish and Wildlife Service through the Division of Federal Aid Administrative Funds under Grant Agreement 1448-98210-98-G027. Funding, in the amount of $220,899, covered the period 1 June 1998 through 31 May 2002. FMS, in turn, subcontracted with researchers at Auburn University and Mississippi State University to conduct the research. Dr. John Grizzle and Dr. L. Esteban Miranda were principle investigators at AU and MSU, respectively. Theodore Henry received a Ph.D. for his work on the project at Auburn University. Mr. Henry's doctoral dissertation serves as the portion of the final report from Auburn University. Mr. Henry intends to submit each chapter as a stand alone publication. Chad Dolan received a MS degree from Mississippi State University for his contribution to the project. A series of six manuscripts (all of which have been accepted and/or submitted for publication) serve as the final report from Mississippi State University.

FMS would like to commend the principle investigators and their students on the outstanding work done to complete this project. FMS is also indebted to Dave Morgan, Gary Reinitz, Tim Hess, and Mary Jones of the Division of Federal Aid for assistance in administering the grant.

Jeff Boxrucker
Past-President
Fisheries Management Section
American Fisheries Society

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The electric fields generated by electrofishing equipment can injure fish; however, the electrical and biological variables important in determining injury are not well understood. Objectives of this study were to define electric-field characteristics of electrofishing boats and to evaluate the effects of similar electric fields on fish in relation to species and stage of development. The overall objective was to provide recommendations for reducing electroshocking-induced injury of fish.

For seven electrofishing boats, voltage gradients were heterogeneous around the boat and ranged from 2.1-3.4 V/cm within 1 m of the bow of the boat to 16-20 V/cm within 5 cm of the anode droppers. Three boats had malfunctioning equipment indicating the need to test electroshocking equipment routinely.

In laboratory experiments with homogeneous electric fields, survival of fish was related to species, stage of development, and electric-field characteristics. Embryos of largemouth bass *Micropterus salmoides* and bluegill *Lepomis macrochirus* had low survival rates after exposure to DC, but PDC had little or no effect on survival. Channel catfish *Ictalurus punctatus* embryo survival was reduced by both DC and PDC and were more susceptible than either largemouth bass or bluegill embryos. Newly transformed juvenile fish was the developmental stage with the highest immediate (1 h) mortality after electroshock, and no delayed (5 d) mortality occurred. Mortality increased with voltage gradient, pulse frequency, pulse width, and duration of exposure. At constant electrical power, mortality of newly transformed juvenile fish was highest when electroshocked in water of 72-230 μS/cm ambient conductivity. Largemouth bass mortality in the laboratory did not differ from mortality induced around an electrofishing boat in a pond when voltage gradients were similar. Blackbanded darters *Percina nigrofasciata* were more susceptible to electroshocking-induced mortality than 9 other species, while newly transformed juvenile paddlefish *Polyodon spathula* and adult western mosquitofish *Gambusia affinis* were most resistant. Grossly visible injuries were observed in fewer than 3% of fish that were electroshocked, but 27-67% (depending on species) had injuries detected during histological examination. Lesions described by histopathology included: necrosis of skeletal muscle, vertebral injury, notochord hernia, and hemorrhage. Results indicate that fish early life stages can be injured during electroshocking; however, electrofishing procedures can be modified and electric fields can be selected to reduce electroshocking-induced injury.

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Style manual or journal used Transactions of the American Fisheries Society

Computer software used WordPerfect 3.5 for Macintosh

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I. INTRODUCTION

The ability of waterborne electric fields to affect fish behavior has been known for more than 100 years. During this time, electrical equipment has been designed to direct (Sternin et al. 1972), capture (Haskell 1939, 1940), anesthetize (Gaikowski et al. 2001), and kill (Robb et al. 2002) fish. As use of electrofishing to sample fish for assessment of fish populations became more common, efforts were made to improve electrofishing techniques (Witt and Campbell 1959; Novotny and Priegel 1974) and to compare catch results with other capture methods (Sanderson 1960; Reynolds and Simpson 1978). Currently, the use of electrofishing as a sampling technique is being questioned because fish can be injured or killed during exposure to electric fields (Snyder 1995; Nielsen 1998). This concern is matched by the importance placed on electrofishing as sampling tool (Reynolds 1996), which may have little or no effects on fish at the population level (Schill and Beland 1995).

In its simplest form, electrofishing equipment includes an electric power source and electrodes to transmit electrical energy into the water. Modifications of this basic design involve pulsators, which allow operators to vary electrical output and select from current types including AC, DC, and various pulsed DC waveforms (Reynolds 1996). The behavior of electric currents between in-water electrodes conforms to Ohm's Law [$V=IR$; where V = voltage (volts), I = current (amps), and R = resistance (ohms)], and the resistance of water is defined by its conductivity (Reynolds 1996). The electric power source generates a voltage [potential electrical energy between two points (Kane and Sternheim 1988)] between the electrodes, and the electric current [and therefore electric power (P=VI; where P = power (watts); Kane and Sternheim 1988)] is determined by water conductivity and the surface area and distance between electrodes (Novotny and Priegel 1974).

The voltage gradients (V/cm) of in-water electric fields around electrofishing equipment are three dimensional and heterogeneous, and the intensity decreases with distance from electrodes (Kolz 1993). Electric power density (D) at a specific location in the water is defined by the product of the voltage gradient squared and the water conductivity (Kolz 1989). The electric power density to which fish are exposed depends on their location relative to electrodes, and cannot be determined by the total electrical output characteristics of electrofishing equipment.

The first report of fish injury following electrofishing was made by Hauck (1949), and subsequent studies, mostly on salmonids, indicated the potential for electrofishing to injure fish (Pratt 1954; McCrimmon and Bidgood 1965; Hudy 1985; Hollender and Carline 1994). Spinal injuries are the most common type of lesions reported (Snyder 1992), and include compression, misalignment, and fracture of vertebrae, often accompanied by hemorrhage (Reynolds 1996). Fish can receive lethal injuries during electroshocking (Collins et al. 1954), however, spinal injuries and death are not correlated (Spencer 1967a; Hudy 1985; Habera et al. 1996). The number of fish dying from electroshocking has been considered negligible based only on juvenile and adult fish (Hudy 1985; Barret and Grossman 1988; Schneider 1992).

Concern about electrofishing-induced injury was elevated following a study by Sharber and Carothers (1988a) which documented spinal injuries in 44-67% of adult rainbow trout *Oncorhynchus mykiss* during field electrofishing with PDC. Following this report, studies were conducted to evaluate electric current types to reduce injury (Hollender and Carline 1994; Sharber et al. 1994; Dalbey et al. 1996), and moratoriums were placed on the use of electrofishing in some areas (Schill and Beland 1995). Despite recent research efforts on the effects of electrofishing on fish, most studies have considered salmonids, and little is known about how electroshocking affects warmwater fish (Snyder 2000).

The electrical output of electrofishing equipment can be manipulated to produce many different current waveforms, and the potential exists for some current types to reduce electroshocking-induced injury. However, electric currents must also be selected to sample fish efficiency. Sharber et al. (1994) developed a complex pulse system (CPS; 3 DC pulses of 240 Hz delivered at 15 Hz) and indicated that electrotaxis (towards anodes) efficiency of CPS for rainbow trout was similar to 30-, 60-, and 512-Hz PDC, but rates of spinal injury were <10% for CPS and increased with pulse frequency to 62% for 512 Hz. The CPS waveform offers some potential for reducing electrofishing-induced injury under some conditions; however, more testing is required. Direct current has been recommended for electrofishing because fish injury can be reduced (Reynolds 1996), but some evidence suggests that mortality of embryos is greater after exposure to DC electric fields than to PDC (Dwyer et al. 1993).

In the present study we examined the relation of fish species, developmental stage, and electric field characteristics on the susceptibility of fish to electroshocking-induced injury. We determined the in-water voltage gradients during normal boat electrofishing operations (Chapter II, Henry et al., in press), and designed equipment to produce homogeneous electric fields with similar voltage gradients in laboratory tanks. The laboratory tanks were used as exposure chambers to investigate the effects of electric fields on fish. We spawned largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and channel catfish *Ictalurus punctatus* and evaluated the effects of electroshocking on survival and premature hatching of embryos (Chapter III) and determined the susceptibility of posthatching developmental stages of the same species and Nile tilapia *Oreochromis niloticus* to electroshocking-induced mortality (Chapter IV). We then identified electric field characteristics that were important in electroshocking-induced mortality of newly transformed juvenile largemouth bass, bluegill, channel catfish, and black crappie *Pomoxis nigromaculatus*; and compared species susceptibility to 60-Hz PDC electroshocking-induced mortality for largemouth bass, bluegill, channel catfish, black crappie, Nile tilapia, rainbow trout *Oncorhynchus mykiss*, paddlefish *Polyodon spathula*, striped bass *Morone saxatilis*, western mosquitofish *Gambusia affinis*, and blackbanded darter *Percina nigrofasciata* (Chapter V). The effect of water conductivity on electric field characteristics and mortality of newly transformed juvenile largemouth bass, bluegill, and channel catfish (Chapter VI) were evaluated, and then we examined electroshocking-induced injuries in newly transformed juvenile fish (Chapter VII). Our objective was to determine the effects of electroshocking on fish (primarily early life stages) and to provide recommendations to reduce the negative effects of electroshocking.

II. COMPARISON OF IN-WATER VOLTAGE GRADIENTS PRODUCED BY ELECTROFISHING BOATS

Abstract.-The voltage gradients of electric fields produced by electrofishing boats are important in determining sampling efficiency and the potential for injuring fish. We evaluated 10 electrofishing boats and found that 3 boats had malfunctions that could impact sampling or operator safety. The in-water voltage gradients were measured for the remaining 7 boats to make comparisons among boats and to determine the voltage gradients present during electrofishing. For all boats evaluated, the cathode was the aluminum boat hull, and the 2 anode arrays each consisted of 3-11 droppers (cables, chains, or rods; 0.6-1.2 m long) suspended from a boom in front of the boat. A grid (1.5 X 2.0 m) was attached to the anode support booms between the anodes and the bow of the boat; this grid facilitated measurements of voltage gradients in the portion of the electric field where most fish are captured. For 9 locations defined by the grid and for 3 water depths (0.1, 0.5, and 1.0 m), a voltage gradient vector was calculated from the horizontal and vertical voltage gradients measured with a probe connected to an oscilloscope. With applied voltages of 900-1000 V, the mean voltage gradient for sampling locations within 1 m of the bow was 2.6 V/cm (SE, 0.1); means for individual boats ranged from 2.1 to 3.4 V/cm. In addition to measurements at locations defined by the grid, maximum voltage gradients of 16-20 V/cm were measured within 5 cm of anode droppers. Despite differences in equipment,

the electrofishing boats produced electric fields with similar voltage gradients when measured at similar locations relative to the electrodes.

Introduction

Electrofishing boats are commonly used to capture fish from rivers and lakes, and the susceptibility of fish to capture is dependent on the electric field intensity and type of electric current (Bohlin et al. 1989). Differences in the electric fields among boats can lead to biased catch rates (Heidinger et al. 1983), and if some boats have electric fields of abnormally high intensity, higher rates of fish injury are possible. Comparison of the electric field intensity among different electrofishing boats is important to assure quality of data. However, we are not aware of any published studies conducted to evaluate and compare intensity of in-water voltage gradients among various electrofishing boats.

Measurement of the electric field generated by an electrofishing boat requires in-water determination of voltage gradients (V/cm) at specific locations in the water (Kolz 1993). A control unit (pulsator) is typically used on electrofishing boats to regulate voltage and current: however, the switches and meters on pulsators indicate the electric output rather than information about the in-water electric field. The waterborne electric field can not be determined simply from the pulsator settings because the electric field is affected by several variables, including water conductivity and the size and shape of electrodes (Novotny and Priegel 1974; Kolz 1993). The electric field is never homogeneous around the boat, and in-water voltage gradients decrease as distance from electrodes increases (Bohlin et al. 1989; Kolz 1993).

Although the arrangement of electrodes used on electrofishing boats can vary, the cathode is usually located close to the boat or the aluminum boat hull itself acts as the cathode, and electrode support booms are used to suspend the anode in front of the boat (Reynolds 1996). A 60-Hz, 110 or 220 volt alternating current (AC) generator is typically used, and selection of generator capacity is dependent on the water conductivity range where electrofishing is to be conducted [i.e., high or low conductivity requires more power (Reynolds 1996)].

Our objective was to evaluate the voltage gradients, generated in a defined region between the electrodes, produced by electrofishing boats. Our approach was to determine the voltage gradient produced by each boat to enable comparison among different boats and electrode configurations. In addition, tests were conducted to determine if electrofishing equipment on each boat was operating correctly, particularly regarding safety of the operator.

Methods

Ten electrofishing boats (4.5-5.5 m length) were considered in this study: 5 boats were evaluated at the Auburn University Fisheries Research Station (Auburn, Alabama), and 5 boats were evaluated at the Walton Fish Hatchery (Georgia). All of the boats had the aluminum boat hull as the cathode and suspended anode droppers from rings or umbrella arrays (Smith-Root Incorporated, Vancouver, Washington) attached to paired electrode support booms extending in front of the boat (Table II-1). Boats used either model VI-A or model GPP Smith Root pulsators (Smith-Root, Vancouver, Washington).

Each boat was evaluated for malfunctions in electric current output. An oscilloscope (Tektronix, Model 720 A, Wilsonville, Oregon) was used to determine if the pulse frequency of the output current was consistent with the pulsator setting, and to determine if the shapes of the output pulses were consistent with half and full wave, rectified AC as specified by the manufacturer (Smith-Root, Vancouver, WA). To determine if the anode array on each electrode support boom received the same electrical energy from the pulsator, the oscilloscope was connected to the boat hull and each anode array individually, and the voltage was measured. At the same time anode arrays were tested, the foot pedal was checked to determine if a voltage was present when the pedal was not depressed. Three boats had malfunctioning electrofishing equipment and were not further evaluated.

Boat electric fields were measured in ponds with ambient water conductivity of 45 µS/cm and temperature at 15-17°C (Yellow Springs Instruments, Yellow Springs, Ohio). Pulsator settings were: pulse frequency, 60- or 120-Hz PDC; voltage level, 900-1000 V; and pulsator current meter indication, 4-6 amps. Water depth was 3-4 m during measurements. An oscilloscope was connected to a probe used to measure the in-water voltage gradient. The sampling probe consisted of two wires separated by 1 cm in the horizontal plane, and two wires separated by 1 cm in the vertical plane. Each wire was insulated except for the terminus of the wire. Two electrically isolated channels of the oscilloscope allowed simultaneous measurement of the peak voltage potential between the two wires in each plane. At each location and depth, mean horizontal (H) and vertical (V) voltage gradients were determined from 2 or 3 replicate measurements, and then a peak voltage gradient vector (E) was calculated as follows:

$$(1) \qquad E = (H^2 + V^2)^{1/2}$$

Because the intensity of the voltage gradient measured in the water is dependent on the orientation of the sampling probe, we conducted preliminary measurements to determine the optimum electrode orientation for sampling the maximum horizonal component of the voltage gradient. We determined that the measured horizontal voltage gradient was within 10% of the maximum when the gap between the two wires measuring the horizontal field gradient was oriented parallel to the centerline of the boat; therefore, this orientation of the probe was used for all electric field measurements recorded.

The maximum intensity of the electric field was measured for 5 boats by approaching an anode dropper (closest to boat) with the sampling probe until the maximum voltage gradient was recorded. Measurements at different depths at specific locations were facilitated by a grid (1.5 X 2.0 m) mounted on the electrode support booms between the anodes and the boat hull (Figure II-1). Measurements were taken at three depths (0.1, 0.5, and 1.0 m) at each of the sampling locations, and voltage gradient vectors were calculated for each depth at each location. For 7 of the electrofishing boats, the grid defined 9 sampling locations between the anode and the cathode; only 6 sampling locations were used for 2 boats because the booms supporting the anodes were short. The grid was set on the electrode support booms 10 cm in front of the prow of the boat, and sampling locations were oriented in rows that were 0.75 m apart (Figure II-1). The distance from sampling locations to the anode droppers differed among boats (Table II-1) because of the variation in length of electrode support booms. Analysis of variance was used to determine if there were significant differences in voltage gradients among boats or at different depths (SAS Version 6 software, SAS Institute, Inc., Cary, North Carolina).

Results

Malfunctions in electrofishing equipment were detected in 3 of the boats tested. One boat produced an electric field (<1.0 V/cm) when the foot pedal was not engaged. In another boat, the 120-Hz PDC setting generated a waveform consisting of one normal pulse followed by a pulse that was lower in intensity (50%) with an abnormal and inconsistent shape. Unequal delivery of electric energy between anode arrays was observed in the third boat.

The maximum voltage gradient vector was determined near the anode for 5 boats and was 16-20 V/cm in all cases. For electric field locations defined by the sampling grid, voltage gradients were highest and most variable at grid locations 1-3, which were the locations nearest the anodes (Table II-2). Because of the short electrode support booms on boats 6 and 7, measurements were collected at only 6 grid locations for these boats (Table II-2). The differences among boats for measured voltage gradients at the grid locations closest to the anodes (locations 1-3) appeared to be primarily related to the variation in distance between the sample location and the nearest anode dropper. At grid locations further from the anode (locations 4-9), all boats had similar voltage gradients (Table II-2); means for boats ranged from 2.1 to 3.4

V/cm. For the relatively uniform voltage gradient at grid locations 4-9, the mean for all boats for the 3 depths combined was 2.6 V/cm (SE, 0.1). Overall, there was no statistically significant difference for voltage gradient measurements among boats.

Voltage gradients at 0.1 and 0.5 m were similar but there was a small, but statistically significant, decrease at 1.0 m. The relative contribution of the horizontal and vertical components to the voltage gradient also varied with depth. The horizontal voltage gradient was higher than the vertical voltage gradient for all grid locations when measured at 0.1 m depth, but at 1.0 m the vertical voltage gradient was higher than the horizontal gradient at grid locations 1-3 (Figure II-2).

Discussion

Although electrofishing has been widely used for collection of fish, no previous studies have compared the in-water voltage gradients produced by various electrofishing boats. For the boats in our study, most measured voltage gradients were similar among boats for the region between the anodes and cathode. The variation in measured voltage gradients at grid locations near anodes was probably related to differences in electrode support boom length, which affected the position of sampling locations in relation to anodes. The region of the electric field measured in our study was small relative to the total electric field area of electrofishing boats, and evaluation of other portions of the electric field is warranted. We measured the voltage gradients in the most intense portion of the electric field because of the potential for fish to be injured by high voltage gradients.

The highest voltage gradients we measured were recorded within 5 cm of anode droppers, but limitations of the measurement technique prevented measurement of a true maximum voltage gradient. Theoretically, the maximum voltage gradient is next to the anode dropper (Novotny and Priegel 1974; Kolz 1993), thus our highest measurements were probably less than the maximum. Measurement of the maximum voltage gradient was difficult because small changes (1 cm) in the position of the sampling probe near the anode resulted in pronounced changes in the voltage gradient. Maximum in-water voltage gradients measured within 5 cm of anode droppers have not previously been reported for electrofishing boats.

Direct measurement of in-water voltage gradients with a sampling probe to measure the horizontal gradient was described by Kolz (1993), but our study was the first to measure vertical voltage gradients and calculate a voltage gradient vector that included both the horizontal and vertical components. In our study the vertical voltage gradient measured at locations close to the anode droppers increased with depth, while at locations near the boat, no relation was observed with depth. Anode droppers extended deeper (up to 1.2 m) than the boat hull cathode, which could explain the depth-related difference in relative vertical voltage gradients near each electrode. During boat electrofishing, fish are exposed to electric fields at depths greater than the 1-m depth we measured, and the vertical component of the electric field could be an important factor determining capture of fish.

The intensity of the electric field produced by boats measured in our study was determined in water with 45 µS/cm ambient conductivity, and changes in field characteristics are expected when boats are operated in different conductivities. If an electrofishing boat is operated at the same voltage level in higher conductivity water, current amperage will increase and the electric power load on the generator will increase (Reynolds 1996). When the electric power demand exceeds generator capacity, the voltage level must be reduced to continue operation. Once in-water voltage gradients are determined for an electrofishing boat at a specific voltage level, they can be related to all other voltage levels [i.e., if the total voltage level is half of the original level, each in-water voltage gradient is also half the original (Kolz 1993)].

When electrofishing is used to collect data for management of fish populations, the quality of the data is dependent on proper functioning of the electrofishing equipment. Defective equipment could alter fishing effectiveness and result in biased data. Eventually, electrofishing equipment requires repair or replacement, and changes in electric field intensity among gear types can impact collection of fish and electrofishing efficiency (Heidinger et al. 1983). Electric fields of electrofishing equipment should be evaluated to determine if equipment is operating correctly.

Evaluation of electrofishing boats should include in-water measurements of voltage gradients at defined locations. After the in-water electric field has been evaluated for a specific boat, periodic checks of the electrical system can be made with the boat out of the water if no modifications are made on the boat. In-water measurements can be conducted with a sampling probe, as described in this study. If a sampling grid is used, it should be positioned relative to the anode droppers (where variation in voltage gradients are highest) rather than to the prow of the boat. The in-water field can also be measured without a grid by sampling at locations near the cathode and each of the anodes. Near the anodes, it is especially important to standardize the distance from the sampling location to the anode if boats are to be compared. Once in-water electric field measurements are determined at a specific total voltage output level, changes in the total voltage output will relate directly to the in-water measurements (Kolz 1993). Then routine measurements of electrofishing equipment can test total voltage output at each anode rather than requiring in-water measurements. When checking the electrical system with the boat out of the water, items to evaluate include operation of the foot pedal and the electrical resistance between each anode and the pulse box, the boat hull, and the generator cathode. The waveform of the pulsator output can also be evaluated with the boat out of the water. Periodic testing of electrofishing equipment is important to safeguard data quality and for operator safety.

Table II-1. Anode characteristics of the electrofishing boats used for comparison of in–water voltage gradients.

Boat	Boom length (m)	Diameter of ring (m)	Dropper number	Dropper length (m)	Type of dropper	Distance to grid[a] (m)
1	3.0	0.8	4	1.2	cable	0.7
2	3.2	0.9	6	1.0	cable	0.8
3	3.3	0.6	8	0.6	cable	1.1
4	2.6	0.6	3	0.9	chain	0.8
5	2.2	0.9	10	1.0	rod	0.3
6	1.7	0.9	11	1.0	rod	0.5
7	2.0	1.0	8-10	1.0	rod	0.9

[a]Distance from the nearest dropper to the grid locations nearest the anodes.

Table II-2. In-water voltage gradients (V/cm) measured at a water depth of 0.5 m. The numbered locations were defined by a grid (Figure II-1). Voltage gradients for each boat were measured 2-3 times at each location and SD is given in parentheses.

		Boat						
Location[a]		1	2	3	4	5	6[b]	7[b]
1	H	2.8(1.9)	3.7(2.1)	3.5(0.1)	5.12(0.1)	12.2(1.1)	5.1(0.4)	3.8(0)
	V	5.0(0.3)	3.0(1.4)	3.0(0)	4.4(0.1)	11.3(0.5)	5.1(0.1)	3.5(0)
	E	5.8	4.7	4.7	6.8	16.6	7.2	5.2
2	H	4.2(0.5)	2.3(0.4)	3.6(0)	2.0(0)	4.4(0)	4.3(0.4)	2.6(0)
	V	4.3(0.2)	2.2(0.5)	2.8(0.3)	2.1(0.1)	4.9(0.3)	4.0(0.4)	2.5(0.1)
	E	6.0	3.2	4.6	2.9	6.6	5.9	3.6
3	H	8.2(4.0)	8.1(1.8)	4.3(0)	6.6(0.3)	5.3(0.1)	3.9(0.1)	2.2(0.3)
	V	5.5(4.6)	5.4(0)	3.5(0)	5.8(0.3)	5.2(0.1)	3.7(0.1)	2.3(0.2)
	E	9.9	9.7	5.6	8.76	7.5	5.4	3.2

(continued)

Table II-2. Continued.

		Boat						
Location[a]		1	2	3	4	5	6[b]	7[b]
4	H	3.3(1.3)	3.2(0.3)	2.0(0.2)	2.2(0.3)	3.0(0)		
	V	2.5(0.6)	2.4(0)	1.4(0.2)	1.7(0.1)	2.6(0.2)		
	E	4.1	4.0	2.4	2.8	3.9		
5	H	3.2(0.6)	2.5(0.4)	2.4(0.2)	2.0(0)	2.8(0.3)		
	V	2.3(0.6)	2.2(0.1)	1.7(0.2)	1.6(0)	2.4(0)		
	E	3.9	3.3	3.0	2.6	3.7		
6	H	3.6(0.2)	2.5(0.1)	2.4(0.1)	2.4(0)	2.0(0)		
	V	2.5(0.1)	2.0(0.1)	1.7(0.1)	2.0(0.1)	1.9(0)		
	E	4.4	3.2	2.9	3.1	2.8		
7	H	2.4(0.3)	1.7(0.4)	1.6(0.2)	1.4(0)	1.4(0)	2.0(0)	1.8(0)
	V	1.4(0.2)	1.3(0.6)	0.9(0.2)	0.9(0)	1.1(0)	1.8(0)	1.4(0.2)
	E	2.8	2.1	1.81	1.7	1.8	2.7	2.3

(continued)

Table II-2. Continued.

		Boat						
Location[a]		1	2	3	4	5	6[b]	7[b]
8	H	2.8(0.2)	2.0(0)	2.4(0.2)	1.9(0.1)	2.0(0)	2.9(0.4)	2.0(0)
	V	1.4(0.1)	1.4(0.2)	1.1(0.3)	1.1(0.1)	1.4(0.1)	2.1(0.1)	1.3(0.1)
	E	3.1	2.4	2.7	2.2	2.4	3.6	2.4
9	H	2.32(0)	1.6(0.3)	1.8(0.1)	1.8(0)	1.4(0)	2.0(0)	1.4(0)
	V	1.3(0.1)	1.0(0.2)	0.8(0.1)	1.0(0)	1.0(0.1)	1.6(0.3)	1.2(0.2)
	E	2.6	1.9	2.0	2.1	1.7	2.6	1.8

[a]H=horizontal gradient, V=vertical gradient, E=combined vector.

[b]Measurements were made at only 6 locations because of the short booms to support the anodes. The rows in this shortened grid were separated by 0.75 m.

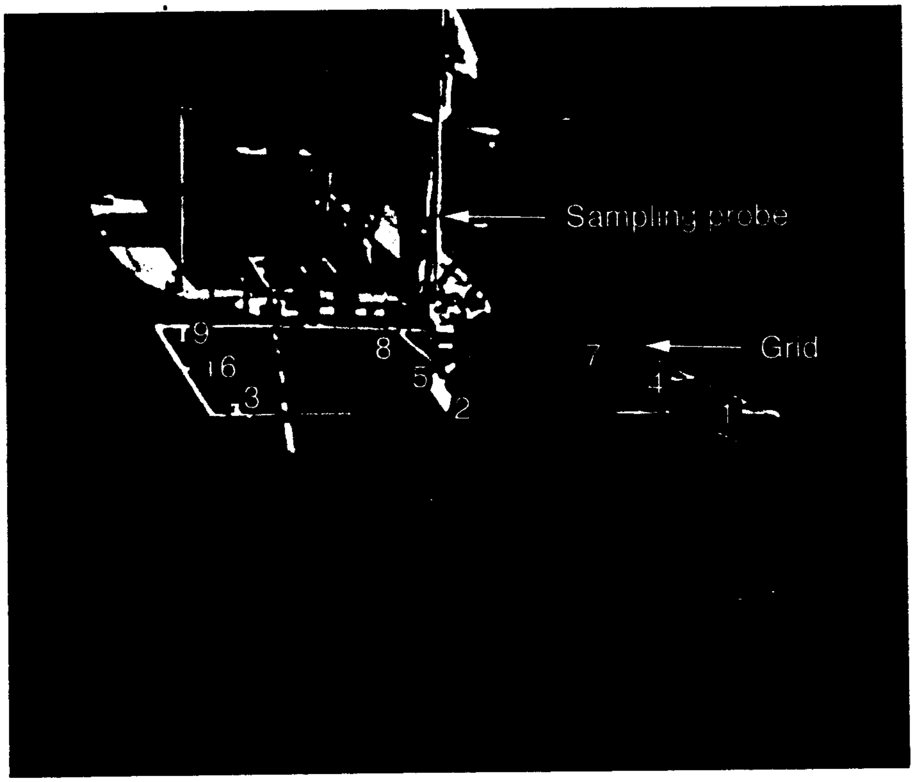


Figure II-1. Electrofishing boat with sampling grid (1.5 X 2.0 m) attached to the electrode support booms. Numbers (1-9) on the grid indicate sampling locations for most boats. The sampling probe measured the voltage gradient at each sampling location at three depths (0.1, 0.5, and 1.0 m).

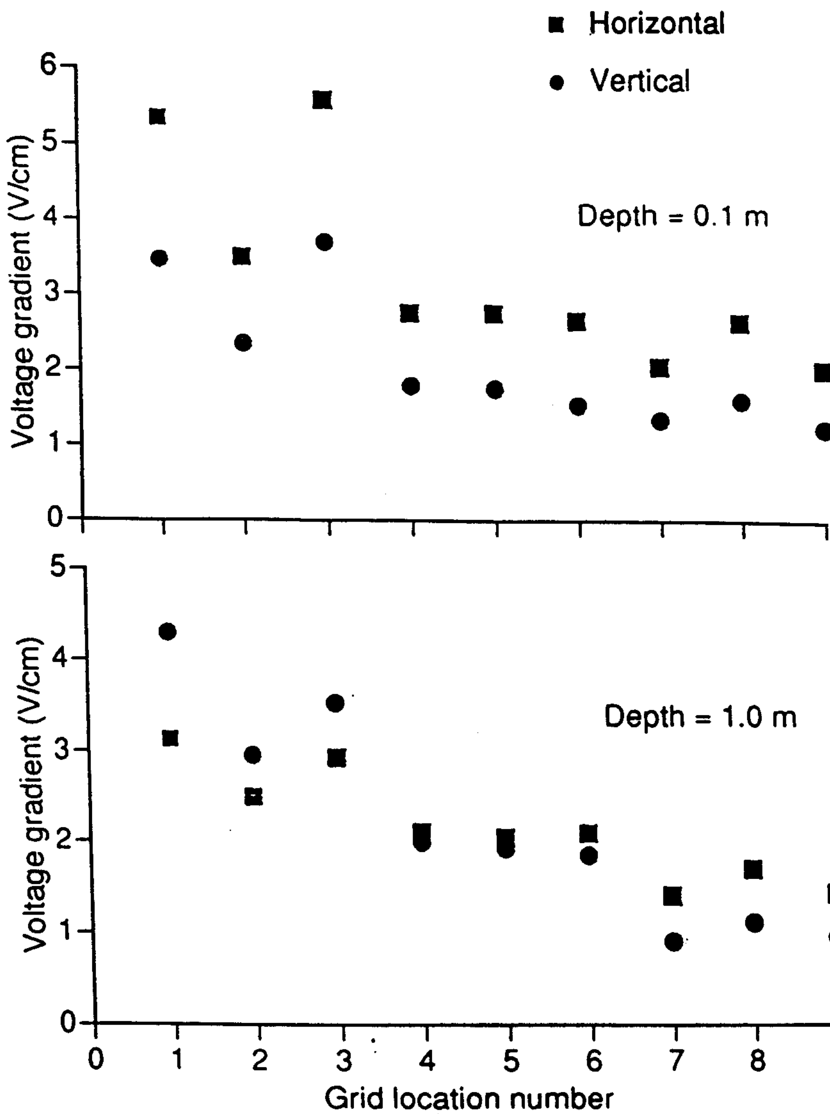


Figure II-2. Mean vertical and horizontal voltage gradients at 0.1 and 1.0 m depths for 7 electrofishing boats (Table II-1). Grid location number indicates the position on the grid (Figure II-1) where the voltage gradients were measured.

III. SURVIVAL AND PREMATURE HATCHING OF SELECTED WARMWATER FISH EMBRYOS AFTER ELECTROSHOCKING

Abstract.-Survival to hatching was determined after electroshocking embryos of largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and channel catfish *Ictalurus punctatus*. Embryos at different developmental stages were exposed for 20 s to homogeneous electric fields (4-16 V/cm) of direct current (DC) or 60- or 120-Hz pulsed direct current (PDC) in water of 100 μS/cm ambient conductivity. For all species, DC reduced survival of embryos at developmental stages before, during, or soon after epiboly; but survival did not differ from controls during later developmental stages. Survival of largemouth bass and bluegill was not reduced by 60- or 120-Hz PDC except for bluegill exposed at 12 h postfertilization. Channel catfish embryo survival was <5% when exposed to PDC at 8 h postfertilization, survival improved for embryos exposed at 67 h for 60 Hz but not 120 Hz, and all embryos exposed to PDC at 150 h postfertilization survived. Exposure durations as short as 5 s resulted in <10% survival of largemouth bass during sensitive stages. Bluegill embryos aged 22 h postfertilization hatched prematurely after exposure to 16 V/cm DC, but survival was not affected. Results indicate that negative effects of electroshocking on embryos of these species can be reduced by avoiding electrofishing when sensitive developmental stages are present. Selection of PDC for electroshocking around largemouth bass and bluegill nests could

reduce negative effects on survival of embryos of these species; however, PDC can reduce survival of channel catfish embryos.

Introduction

Fish embryos are not targets of electrofishing; however, embryos can be exposed to electric fields during routine electrofishing operations. Electrofishing during the spawning season, when fish are concentrated in shallow spawning areas, can increase the likelihood of exposing fish embryos to electric currents. For the species that have been studied, including salmonids (Godfrey 1957; Dwyer et al. 1993; Dwyer and Erdahl 1995; Roach 1999), walleye *Stizostedion vitreum* (Newman and Stone 1992), and razorback suckers *Xyrauchen texanus* (Muth and Ruppert 1997), electroshocking has been shown to reduce survival of embryos under some conditions. However, the effects of electroshocking on embryos of warmwater species have not been investigated.

Fish embryos can be exposed to high voltage gradient electric fields during electrofishing operations. Voltage gradients up to 20 V/cm can be produced near (<5 cm) anode droppers of electrofishing boats (Henry et al., in press), and electrodes can pass in close proximity to fish embryos during electrofishing operations. For fish embryos concentrated in nests, the negative effects of electrofishing could possibly affect the entire brood during a single exposure.

Reduced survival of embryos after electroshocking has been related to the stage of embryonic development and the electric field characteristics during electroshocking (Godfrey 1957; Newman and Stone 1992; Dwyer and Erdahl 1995; Muth and Ruppert 1997; Roach 1999; Cho et al 2002). Results indicate that embryo survival could be improved by reducing voltage gradient (Godfrey 1957: Dwyer and Erdahl 1995; Muth and Ruppert 1997), reducing frequency of pulsed direct current (PDC) (Muth and Ruppert 1997), and limiting exposure duration (Godfrey 1957). For warmwater fish embryos, understanding the impact of electroshocking on survival is necessary to provide recommendations to modify electroshocking procedures to reduce the negative effects of electrofishing if necessary.

Our objectives were to determine if survival of warmwater fish embryos was affected by electroshocking, to determine which electric field types impacted survival, to determine the developmental stages that had lowest survival after electroshocking, and to evaluate premature hatching after electroshocking. Experiments were conducted with embryos of largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and channel catfish *Ictalurus punctatus*.

Methods

Experimental fish.-Eggs were obtained by spawning largemouth bass, bluegill, and channel catfish at the Auburn University Fisheries Research Station, Auburn, Alabama. Adult largemouth bass (15 males and 15 females) were stocked into a pond (200 m^2; alkalinity, 28 mg/L as $CaCO_3$; hardness, 30 mg/L as $CaCO_3$; temperature, 19-22°C), and synthetic fiber mats (35 X 35 cm) were provided for spawning substrate on the mud bottom (0.4-1.3 m deep). Adult bluegill (2 females and 1 male) were stocked in a fiberglass tank (2400 L) with flow-through (2 L/min) pond water (same as above except temperature was 25-27°C) and two 6-L plastic bowls each containing 5-10 rocks (≈1.0 cm diameter) were used for spawning substrate. Adult channel catfish were stocked in cages (2 females and 1 male) in ponds (400 m^2), and each cage had an enclosed spawning container. Water quality for channel catfish ponds was the same as for bluegill.

Spawning mats, bowls, and containers were checked twice each day, and when spawning activity was observed, containers were checked more frequently and the time of fertilization was estimated when eggs were found. Channel catfish egg masses were separated by immersion in a sodium sulfite solution (1.5% Na_2SO_3; Ringle 1992), and the same solution was used to facilitate removal of bluegill eggs from the bowl surface. Largemouth bass eggs did not adhere to the mat and were removed by gently agitating the mat in a 20-L plastic bucket.

Electric fields.-Electric fields were generated by an electrofishing pulse box (Coffelt, TEG-10 Proto 1, Flagstaff, Arizona), modified to be powered by standard 110 V AC and to produce DC and square-pulsed DC (PDC) electric fields. Exposure chambers were plastic troughs (38.1 X 7.7 cm, and 5.1 cm deep) and electricity was delivered to the water with aluminum plate electrodes. The electrodes conformed to the cross-sectional area of the troughs, were separated by 30 cm, and could be moved from trough to trough. Electric fields were measured with an oscilloscope each time a fish was exposed (Tectronix THS 720A, Beaverton, Oregon). The oscilloscope was used to measure voltage amplitude, pulse frequency, and pulse width; and all electrical measurements of experimental treatments were within 3% of the expected value. The voltage amplitude was used for determining the voltage gradient. Ambient water conductivity was 96-104 μS/cm in all experiments; water conductivity was adjusted by adding artificial sea salts (Instant Ocean Synthetic Sea Salt, Mentor, Ohio). Prior to experiments, homogeneity of the electric field was tested with the oscilloscope connected to a sampling electrode (Henry et al., in press), and voltage gradients were found to be uniform throughout the exposure chambers.

Experimental design.-Each species was evaluated in separate experiments, and the number of eggs stocked (by plastic pipet) in troughs varied among species (see below). In each experiment, eggs were obtained from a single spawn. Each of the 61 troughs (described above) had flow-through (15-20 mL/min) water exchange (well water; alkalinity, 24 mg/L; hardness, 25 mg/L; pH, 6.9; and oxygen, > 6.0 mg/L) and plastic covers. Temperature was maintained at 20.9-23.4°C for largemouth bass, 27.3-28.1°C for bluegill, and 26.9-27.9°C for channel catfish. After stocking, eggs in each trough were individually examined, counted, and dead eggs were removed. No other handling of eggs occurred during experiments. Eggs of all 3 species were treated with 1500 mg/L formalin (trough concentration) within 15 min of stocking, and water flow was restored to the trough after 15 min. For channel catfish eggs, the same formalin treatment was repeated every 24 h.

The electric fields tested included DC, 60-Hz PDC, or 120-Hz PDC at voltage gradients of 4-16 V/cm. The pulse width of PDC was 3 ms. Three randomly selected troughs containing embryos were used as replicates for each experimental treatment. An experimental treatment consisted of a specific electric field type, and exposure (20-s duration) of embryos occurred only once. At the preselected time postfertilization, water flow to the trough was turned off, electrodes were placed in the trough for electroshocking, then electrodes were removed and water flow was restored within 1 min. During each experiment, three troughs were randomly selected to serve as controls. Treatment of control embryos consisted of stopping the water flow for 1 min and placing the electrodes in the trough without energizing the electrodes. Experiments ended when eggs hatched or were obviously dead. Newly hatched larvae were siphoned from each trough and preserved in 10% neutral buffered formalin and counted. Survival was calculated by dividing the number of newly hatched larvae by the number of eggs stocked in the trough at the beginning of the experiment.

Bluegill embryos (70-200/trough) were exposed to electric fields at 7-23 h postfertilization and largemouth bass embryos (70-300/trough) were exposed at 10-42 h postfertilization. An additional experiment was conducted with largemouth bass embryos to evaluate the effect of exposure duration (5, 10, and 20 s) to 8 V/cm DC, and embryos were exposed at 16, 21, and 26 h postfertilization. Simulated electroshocking of control embryos of bluegill was at 9-11 h postfertilization and for largemouth bass was at 16-22 h postfertilization.

Two experiments were conducted to determine the survival of channel catfish embryos after electroshocking. In the first experiment, embryos (150-300/trough) were exposed from 8-92 h postfertilization (as described above), and the electric fields tested were: DC (4-16 V/cm) or 60- to 120-Hz PDC (16 V/cm). For the second experiment, channel catfish embryos aged 148 h postfertilization were stocked into troughs (10-15 embryos/trough) and exposed to a specific electric field [DC or PDC (60 or 120 Hz) at 16 V/cm] at 150 h postfertilization. Simulated electroshocking of control embryos was at 18 h postfertilization in the first experiment and at 150 h postfertilization in the second experiment.

In addition to control troughs, 3 troughs during each experiment were randomly selected for sampling embryos for determination of developmental stage, and the embryos in these troughs were not exposed to an electric field. Embryos (10-50) were sampled from these troughs each time postfertilization that shocking occurred in other troughs, and embryos were preserved in 10% neutral buffered formalin. After fixation, the chorion was removed by dissection and embryos were stained with borax carmine (Humason 1979). Description of the stage of development of fish embryos was conducted after observation with a dissecting microscope.

The susceptibility of embryos to premature hatching induced by electroshocking was evaluated for bluegill. At 22 h postfertilization, bluegill embryos were exposed to 16 V/cm DC for 20 s, and 15 embryos were immediately pipetted into a glass dish (10 cm diameter, 2 cm deep) containing water from the troughs. Three replicate troughs were used, and an additional 3 dishes contained control embryos exposed to the same handling but not to electroshock. Hatching and behavior of embryos was monitored with a dissecting microscope, and after 35 min, embryos were returned to troughs and survival was evaluated for 5 d (through depletion of yolk sac).

Statistics.-For all experiments, survival data was arcsin transformed before statistical analysis (Zar 1984). Data were analyzed by analysis of variance (ANOVA) to compare mean survival among electric treatments and with controls. Significant differences among means were identified by Tukey test at the 0.05 probability level (SAS version 8.2, SAS Institute, Cary, North

Carolina). In most cases, results of embryo survival are presented as "percent of control" (A), which was calculated by:

$$A = (100)(\text{mean survival of control})^{-1}(\text{survival of treatment}) \qquad (1)$$

Results

Hatching occurred at 26-30 h postfertilization for bluegill, 45-50 h for largemouth bass, and 160-175 h for channel catfish (Table III-1). For bluegill, by 15 h postfertilization the blastoderm covered the yolk, and by 18 h, movement of the embryo was observed. For largemouth bass, the blastoderm covered the yolk between 17 and 29 h, and movement of the embryo was observed at 29 h. The yolk of channel catfish was covered or nearly covered by the blastoderm by 32 h postfertilization and movement of the embryo was observed by 67 h.

At developmental stages when movement of the embryos could be observed; embryos of all species moved rapidly within the chorion during the initial 5 s of electroshocking, movements generally ceased during the remaining 15 s, and movements of the embryo returned to preshocking levels within 5 min after the end of electroshocking. No deformities of hatched larvae were observed among electroshock treatments or controls.

Survival was lower ($P < 0.05$) for largemouth bass embryos exposed to 8 V/cm DC at 10, 17, or 29 h postfertilization than for embryos exposed to 60- or 120-Hz PDC or for unshocked embryos (Figure III-1). Bluegill embryos younger than 15 h postfertilization had significantly ($P < 0.05$) lower survival when exposed to 16 V/cm DC than to either 60- or 120-Hz PDC or unshocked controls. Lower voltage gradients of DC (4 V/cm for largemouth bass; 8 V/cm for bluegill) did not reduce survival of embryos relative to controls at any developmental stage. No significant ($P > 0.05$) differences in embryo survival were detected between 60- and 120-Hz PDC at any time; therefore, results were combined (Figure III-1). For largemouth bass, PDC did not affect embryo survival at any time; however, for bluegill embryos exposed to either 60- or 120-Hz PDC at 12 h postfertilization, survival was significantly ($P < 0.05$) lower than control embryos.

At 16 h and 21 h postfertilization, survival of largemouth bass to 8 V/cm DC was < 10% of control and did not differ ($P > 0.05$) for exposure durations of 5, 10, or 20 s (Figure III-2). Survival of largemouth bass embryos decreased with exposure duration when embryos were exposed at 26 h postfertilization, and a 5-s exposure did not reduce survival relative to unshocked embryos.

Exposure of channel catfish to 8 V/cm DC when younger than 88 h postfertilization resulted in significantly ($P < 0.05$) lower survival than for unshocked embryos (Figure III-3). At 8 h postfertilization, survival was less than 5% of control for all embryos exposed to DC at all voltage gradients (4-16 V/cm); however, at 67 h postfertilization, survival of embryos decreased significantly with voltage gradient. For 60- and 120-Hz PDC (16 V/cm), survival of channel catfish embryos was <5% of control when embryos were exposed at 8 h postfertilization; and at 67 h postfertilization, survival of embryos was significantly lower for 120 Hz than for 60 Hz, which did not differ from the control (Figure III-4). All channel catfish embryos exposed to PDC at 150 h postfertilization survived.

Premature hatching was induced in bluegill embryos exposed at 22 h postfertilization (Figure III-5). Within 15 min after exposure to 16 V/cm DC, a bulge developed in the chorion surrounding the bluegill embryo, the chorion became transparent in the area of the bulge, and within 30 min the embryo hatched. Little movement of the bluegill embryo was observed after exposure to electricity; movements of the shocked and control embryos were similar. Unshocked embryos exposed to the same handling did not hatch until 26-30 h postfertilization. Survival of prematurely hatched embryos for 5 d (through depletion of yolk) did not differ from unshocked controls.

Discussion

Although fish embryos are not targets of electrofishing, they are exposed to electric fields during electrofishing. For each species in this study, DC electric fields resulted in low survival during the first half of embryonic development. Direct current electric fields are generally considered the least injurious current type for collecting juvenile and adult fish, and use of DC has been suggested as a means to minimize electrofishing-induced injury (Reynolds 1996). However, the present study indicated that use of DC should be carefully evaluated if electrofishing is planned to coincide with the presence of sensitive stages of fish embryos. This is of particular concern for endangered species that spawn in concentrated areas and where the use of electrofishing could have population level effects.

Embryo survival after electroshocking was related with the stage of embryonic development, which was consistent with previous studies with other species of fish (Godfrey 1957; Newman and Stone 1992; Dwyer et al. 1993; Muth and Ruppert 1997). Of the species that have been examined, early developmental stages of embryos were more susceptible to electroshocking than developmental stages near hatching. The stage at early epiboly was most susceptible to electroshock for razorback suckers *Xyrauchan texanus* (Muth and Ruppert 1997). For rainbow trout *Oncorhynchus mykiss*, survival was lowest when embryos were electroshocked on day 8 (10.4°C) postfertilization and corresponded to the developmental period when embryos were most susceptible to physical shock (Dwyer et al. 1993).

Godfrey (1957) suggested that survival of salmonid embryos after electroshocking improved upon reaching the eyed stage because the yolk becomes covered by a protective layer of cells. Epiboly is the period during embryonic development when the blastoderm overgrows the yolk (Warga and Kimmel 1990). In our study, survival improved rapidly for bluegill after completion of epiboly; however, for largemouth bass, epiboly was completed between 17 and 29 h postfertilization, but survival at 29 h postfertilization did not differ from survival at 10 h postfertilization. For channel catfish, epiboly was completed near 32 h postfertilization, but survival of embryos after exposure to 8 V/cm DC did not improve until embryos were older than 50 h postfertilization. Although completion of epiboly could improve survival of fish embryos after electroshocking, other developmental changes also appear to be important.

Our results indicate that reduction in survival of embryos after electroshocking was related to fish species. For DC electric currents, the lowest voltage gradient to reduce survival was 16 V/cm for bluegill, 8 V/cm for largemouth bass, and 4 V/cm for channel catfish. Voltage gradients less than 4 V/cm potentially reduce survival of channel catfish embryos, but were not tested in this study. Voltage gradients of 2.2 V/cm can reduce survival of cutthroat trout *Oncorhynchus clarki* to less than 10% when exposed on day 8 postfertilization (Dwyer and Erdahl 1995). The reasons for the relative differences in susceptibility to electroshocking-induced mortality among species in this study are unknown; however, one explanation could be relative differences in egg size. Cell membranes can be disrupted by electric fields, and larger cells are suspected to be more vulnerable (Gaylor et al. 1988). Channel catfish have larger eggs (Saksena et al. 1961) than largemouth bass eggs, which are larger than bluegill eggs (Hardy 1978).

We found that DC electric fields were more likely to kill largemouth bass and bluegill embryos than PDC, and that survival decreased as voltage gradient increased. Pulsed DC resulted in higher survival than DC for cutthroat trout; however Dwyer and Erdahl (1995) concluded that voltage level was more critical to egg survival than current type. Voltage level has also been reported to be an important factor in survival of razorback suckers (Muth and Ruppert 1997) and Atlantic salmon *Salmo salar* embryos (Godfrey 1957). Our comparison of DC and PDC for channel catfish was inconclusive because survival at 8 h postfertilization was <10% of control for DC and PDC, while at 67 h postfertilization voltage gradients of the DC and PDC differed.

The potential for fish embryos to be exposed to electric fields during electroshocking depends partly on the spawning behavior of individual species. Largemouth bass and bluegill deposit eggs in exposed nests, a characteristic that increases vulnerability to high intensity electric fields that can occur when electrodes pass over nests. Salmonids that bury their eggs in substratum can receive some protection from electroshocking (Godfrey 1957); however, even when buried, embryo survival can be reduced by electroshocking (Dwyer et al. 1993). Channel catfish spawn in cavities, which could prevent embryos from coming in contact with high intensity electric fields near electrodes.

Electric fields have been used to induce premature hatching in various species of fish (Yamagami 1988); however, the potential for electric fields to induce hatching during electrofishing has not been considered. After electroshocking bluegill at 22 h postfertilization, we observed changes in the appearance of the chorion, and embryos hatched 4-8 h prior to hatching of unshocked controls. Observations of premature hatching in medaka *Oryzias latipes* embryos after electric exposure were similar, and hatching was related to release of enzymes from hatching gland cells present on the embryo (Yamagami 1981). Although we found no effect of premature hatching on survival of bluegill in laboratory experiments, premature hatching in the field could result in higher mortality because of increased risks of being swept from the nest by water currents and of increased risk of predation.

The present study indicated that electroshocking can reduce the survival of warmwater fish embryos; however, the potential for electrofishing to have population level effects after exposure of embryos is unknown. For largemouth bass and bluegill, voltage gradients of relatively high intensity (8-16 V/cm) were required before embryo survival was reduced, and while voltage gradients up 20 V/cm can be produced by electrofishing equipment (Henry et al., in press), they only occur in close proximity (< 5 cm) to electrodes. The potential for population level effects will depend on the amount of electroshocking conducted in habitats where spawning is occurring. Although channel catfish embryos were susceptible to voltage gradients of lower intensity than largemouth bass or bluegill, spawning behavior of channel catfish likely reduces potential for embryos to be electroshocked. Our results indicate that if operators of electrofishing equipment avoid electroshocking in habitats containing sensitive developmental stages of fish embryos, negative effects of electroshocking on survival can be avoided. If electroshocking must be conducted when fish embryos are present, operators should consider selecting PDC rather than DC and operating equipment at voltage levels that do not exceed sampling objectives.

Table III-1. Stages of development when embryos were electroshocked. Water temperature was 27.3-28.1°C for bluegill, 20.9-23.4°C for largemouth bass, and 26.9-27.9°C for channel catfish.

Species	Hours PF[a]	Description of developmental stage
Bluegill	7	Blastoderm covered 1/3-1/2 of the yolk
	11	Blastoderm covered 2/3 of yolk, head expanded laterally
	12	Blastoderm covered 2/3-3/4 of yolk
	15	Blastoderm covered yolk, 7-8 rudimentary somites, tip of tail free from yolk
	18	Embryo movement, rudimentary optic ventricle, notochord
	21	Tail extended almost to head, 28 somites
	26-30	Hatching
Largemouth bass	10	Blastoderm covered 1/3 of yolk
	17	Blastoderm covered 3/4 of yolk, embryonic axis visible
	29	Embryo movement, 18-20 somites, notochord and optic vesicles visible, tail free from yolk
	42	Tail extended almost to head, 30 somites
	45-50	Hatching

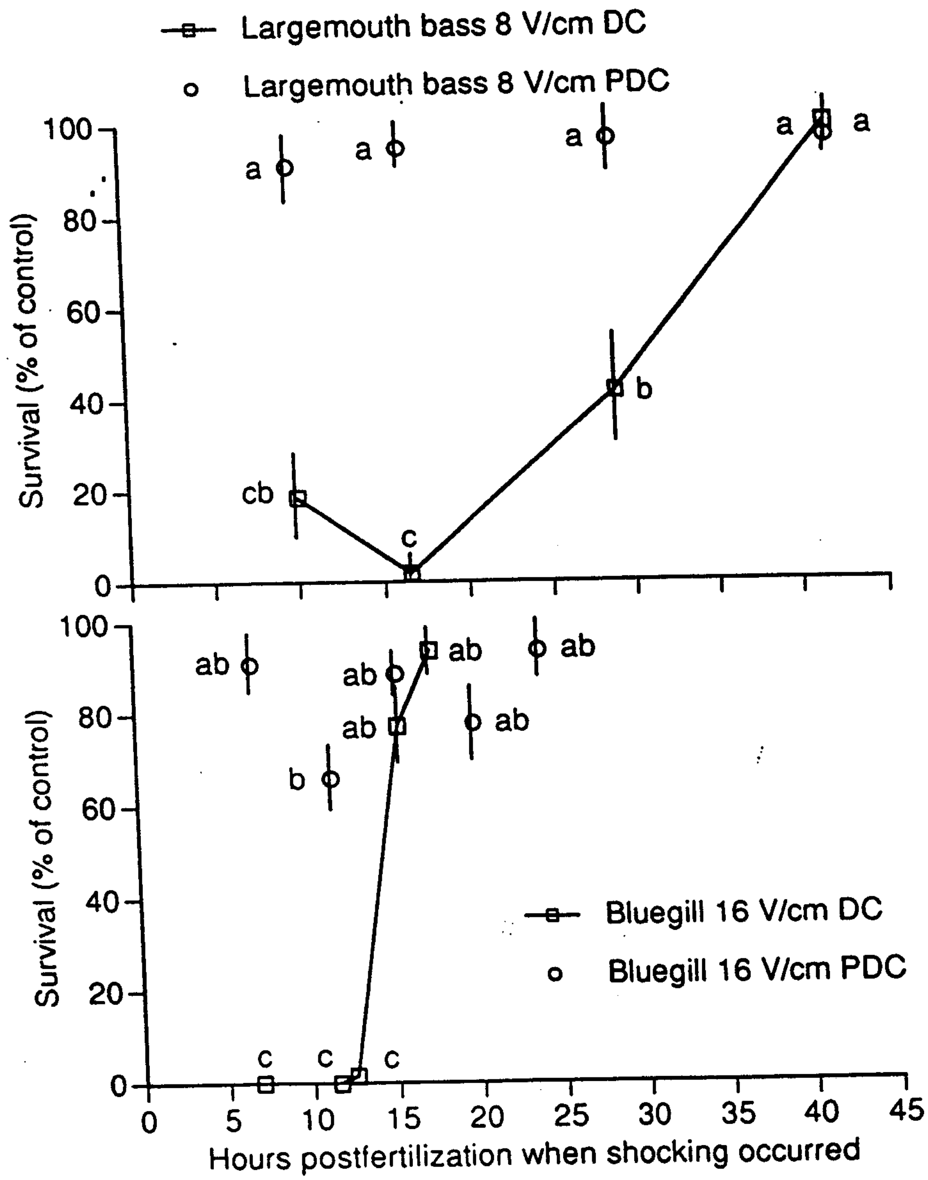
(continued)

Table III-1. Continued.

Species	Hours PF[a]	Description of developmental stage
Channel catfish	8	Blastoderm rounded, projected above yolk, and not yet flattened
	17	Blastoderm covered about 1/4 of the yolk
	32	Blastopore closed or nearly closed, anterior and posterior portions of embryo distinct
	48	Tail extends well off yolk, 28-32 somites, optic and otic vesicles visible
	67	Embryo movement, lens visible in eye, no melanin in eye, rudimentary barbels
	88	Melanin in eye, mouth visible
	160-175	Hatching

[a]postfertilization

Figure III-1. Mean ± SE survival to hatching of largemouth bass and bluegill embryos exposed for 20 s to DC or PDC (60 and 120 Hz) electric fields in water with 96-104 μS/cm ambient conductivity. No significant ($P > 0.05$) differences in survival were observed between 60 and 120 Hz PDC and results were combined for each exposure time. Three replicate troughs were used for each treatment and postfertilization exposure time. Letters next to means indicate significant ($P < 0.05$) differences in mean survival. Within each species, means with the same letter were not significantly different, and means with the letter "a" were not different from the control. Largemouth bass hatched 45-50 h postfertilization, and survival of control embryos was 81 ± 3%. Bluegill hatched 26-30 h postfertilization, and survival of control embryos was 88 ± 7%.


Largemouth bass 8 V/cm DC
Largemouth bass 8 V/cm PDC
Survival (% of control)
100
80
60
40
20
0
a
a
a
a
a
b
cb
c
ab
ab
ab
ab
ab
ab
b
c
c
c
Bluegill 16 V/cm DC
Bluegill 16 V/cm PDC
0
5
10
15
20
25
30
35
40
45
Hours postfertilization when shocking occurred

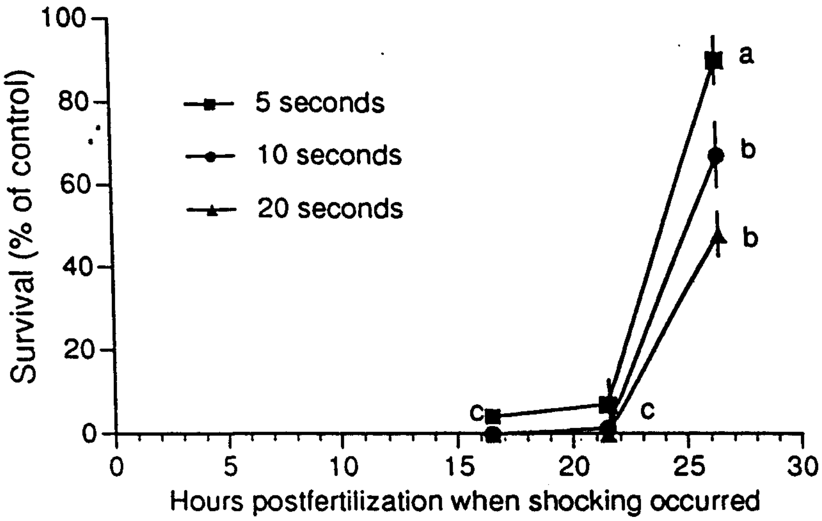


Figure III-2. Effect of electroshock duration (5-20 s) on mean (± SE) survival to hatching of largemouth bass embryos exposed at different times postfertilization to 8 V/cm DC in water of 96-104 μS/cm conductivity. Three replicate troughs were used for each treatment and postfertilization exposure time. Significant ($P < 0.05$) differences among means are indicated by different letters, and means with the letter "a" did not differ from the control. Embryos hatched 45-50 h postfertilization, and survival of control embryos was 69±5%.

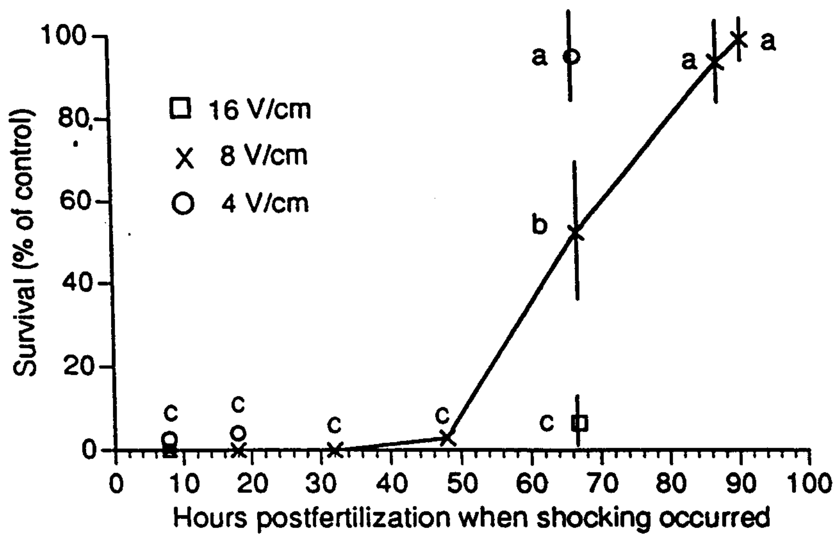


Figure III-3. Mean ± SE survival to hatching of channel catfish embryos exposed for 20 s to 4-16 V/cm DC at different times postfertilization in water of 96-104 µS/cm conductivity. Three replicate troughs were used for each treatment and postfertilization exposure time. Significant ($P < 0.05$) differences among survival means are indicated by different letters, and means with the letter "a" did not differ from the control. Hatching occurred from 160 to 175 h postfertilization and survival of controls was 52 ± 5.2%.

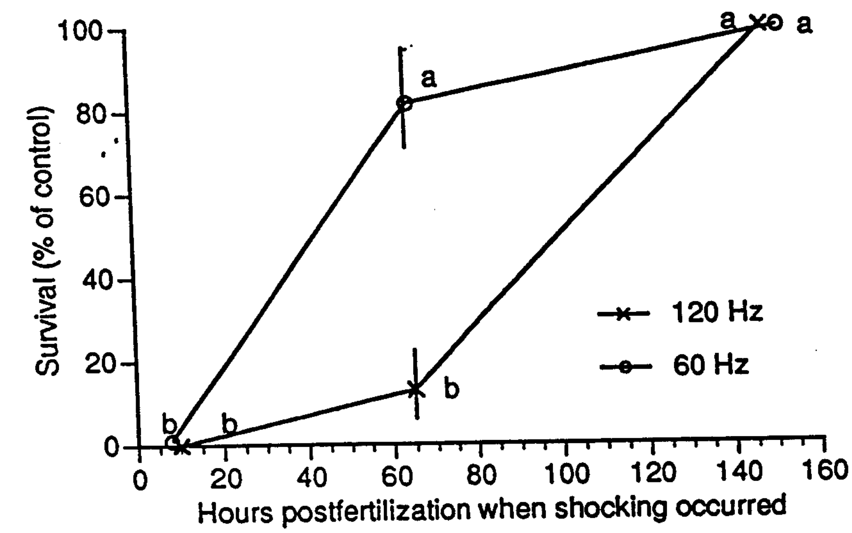


Figure III-4. Mean ± SE survival to hatching of channel catfish embryos exposed for 20 s to 60- or 120-Hz PDC at 8, 67, or 150 h postfertilization in water of 96-104 µS/cm conductivity. Three replicate troughs were used for each treatment and postfertilization exposure time. Embryos exposed at 8 or 67 h postfertilization (experiment 1) were from the same spawn and each trough contained 150-300 embryos. For embryos exposed at 150 h postfertilization (experiment 2), each trough contained 10-15 embryos. Means with the same letter were not significantly ($P < 0.05$) different, and means with the letter "a" were not different from the control. Hatching occurred 160-175 h postfertilization and survival of control embryos in experiment 1 was 52 ± 5.2%, and survival of control embryos was 100% in experiment 2.

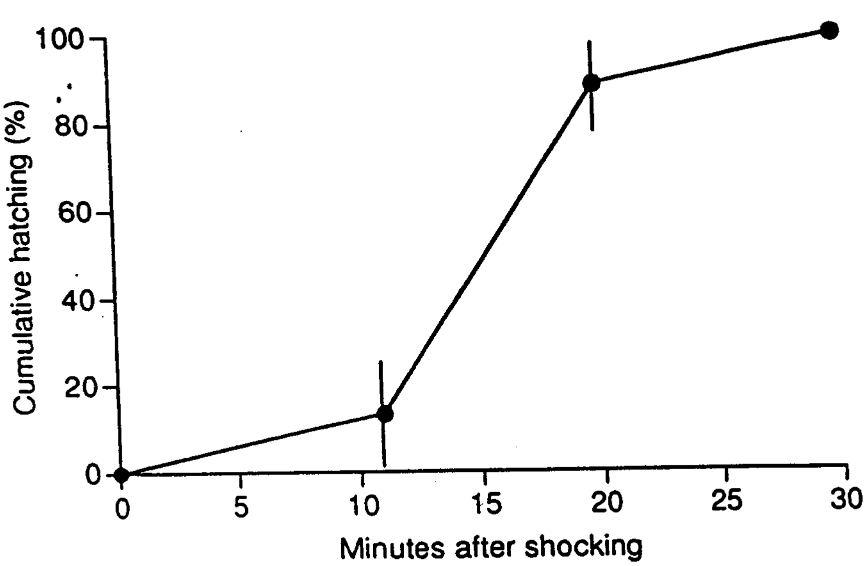


Figure III-5. Percent hatching (mean ± SE) of bluegill embryos hatching after exposure for 20 s to 16 V/cm DC at 22 h postfertilization in water of 96-104 µS/cm. Three replicate groups of 15 embryos were either electroshocked or not electroshocked (controls). Temperature was 27.3-28.1°C and unshocked embryos did not hatch until 26-30 h postfertilization.

IV. ELECTROSHOCKING-INDUCED MORTALITY OF FOUR FISH SPECIES DURING POSTHATCHING DEVELOPMENT

Abstract.-Immediate (1-h) mortality of electroshocked fish was related to the stage of development after hatching. Larvae and juveniles of four species of fish were exposed to homogeneous voltage gradients (2-16 V/cm) of 60-Hz pulsed direct current in water with ambient conductivity of 100 μS/cm. Recently hatched fish and fish older than 100 d did not die following electroshocking. The developmental period most susceptible to electroshocking-induced mortality occurred near the time of transformation from larvae to juveniles for all four species. The highest predicted mortality occurred at 22 d or 19-21 mm total length (TL) after hatching for bluegill *Lepomis macrochirus*, 36-41 d or 29-32 mm TL for largemouth bass *Micropterus salmoides*, 42-43 d or 27-30 mm TL for channel catfish *Ictalurus punctatus*, and 38 d or 21 mm TL for Nile tilapia *Oreochromis niloticus*. No delayed mortality of channel catfish occurred during the 5-d observation period. A field validation experiment with 25-d-old largemouth bass (14-22 mm TL) indicated that mortality in the laboratory or around an electrofishing boat did not differ if voltage gradients were similar.

Introduction

In aquatic habitats where electrofishing is conducted, many species and life history stages of fish can be exposed to electric fields regardless of the objectives of the electrofishing operation. Fish can be injured by electric exposure (Hauck 1949; Reynolds 1996), but there is no information about the relative susceptibility of different life stages of any fish species. Age-related differences in susceptibility to electrofishing-induced injury need to be determined, and if sensitive developmental stages exist, electrofishing procedures should be modified if the negative effects of electric exposure are important.

Previous research on the negative effects of electrofishing on fish can be divided into lethal and sublethal categories. Sublethal effects include impacts on growth (Gatz et al. 1986; Dalbey et al. 1996), disease progression (VanderKooi et al. 2001) and reproduction (Muth and Ruppert 1996), but most commonly, studies have evaluated injuries related to vertebrae and associated tissues (Snyder 1992). Although larvae and newly transformed juveniles have not been considered, in larger fish the frequency and severity of spinal injuries increase with increasing fish length (Hollender and Carline 1994; Dalbey et al. 1996; Thompson et al. 1997a). The importance of spinal injuries on fish survival is unclear, and some studies indicate that grossly visible injury is not related to fish mortality following electrofishing (Spencer 1967a; Hudy 1985; Habera et al. 1996).

Several studies have concluded that only a negligible number of fish are killed during electrofishing and that fish mortality is not a concern (Hudy 1985; Barrett and Grossman 1988; Schneider 1992). Although laboratory experiments indicate that fish size is not directly related to mortality (Collins et al. 1954; Whaley et al. 1978), Habera et al. (1996) found that 7-d mortality was higher for rainbow trout (*Oncorhynchus mykiss*) < 100 mm TL than for larger fish. However, larvae and recently transformed juveniles have not been considered in either field or laboratory studies.

The objective of our study was to evaluate electroshocking-induced mortality of life history stages from yolk-sac larva to juvenile for largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), and channel catfish (*Ictalurus punctatus*). Nile tilapia (*Oreochromis niloticus*) were also evaluated, but developmental stages included only older larvae (transitioning to the juvenile stage) and young juveniles. Additional objectives were to determine if delayed mortality (5 d) occurred after channel catfish were electroshocked, and to compare the mortalities of young largemouth bass in laboratory and field experiments.

Methods

Experimental animals.-To obtain larvae and juveniles for experiments, adult largemouth bass, bluegill, and Nile tilapia were spawned in earthen ponds or fiberglass tanks at the Auburn University Fisheries Research Station, and channel catfish eggs were obtained from a commercial source (Blackbelt Aquaculture, Newbern, Alabama). Eggs were hatched in laboratory aquaria, and young largemouth bass and bluegill were fed zooplankton, brine shrimp (*Artemia*), and fish feed (Ziegler Brothers, Gardners, Pennsylvania). Channel catfish and Nile tilapia were fed fish feed. Fish were kept in 40-L flow-through (0.25 L/min) aquaria receiving well water. The well water had the following characteristics: alkalinity, 25 mg/L as $CaCO_3$; hardness, 23 mg/L as $CaCO_3$; temperature, 18°C; pH, 7.1; dissolved oxygen, >6.0 mg/L; and ambient conductivity, 80 µS/cm.

Older largemouth bass [age, 13 months; 222 mm mean total length (TL); range, 176-300 mm TL; *N*=28] and channel catfish (age, 15 months; 295 mm mean TL; range, 223-370 mm TL; *N*=31) were collected by seining ponds at the Auburn University Fisheries Research Station. Fish were kept for 3-5 d in 2,400-L fiberglass tanks receiving reservoir water (2-3 L/min) and were not fed after capture. The reservoir water had the following characteristics: alkalinity, 30 mg/L as $CaCO_3$; hardness, 33 mg/L as $CaCO_3$; temperature 25°C; pH, 7.1; oxygen, >6.0 mg/L; and ambient conductivity, 70 µS/cm.

Electric fields.-Electric fields were generated by an electrofishing pulse box (Coffelt, TEG-10 Proto 1, Flagstaff, Arizona), modified to be powered by standard 110 V AC and to produce DC and square-pulsed DC (PDC) electric fields. Exposure chambers were plastic troughs (38.1 X 7.7 cm, and 5.1 cm deep) for fish younger than 100 d, and a 40-L glass aquarium for larger fish. Electricity was delivered to the water with aluminum plate electrodes, which conformed to the cross-sectional area of the troughs or glass aquarium. In troughs, the electrodes were separated by 30 cm and could be moved from trough to trough. In the glass aquarium, plates were separated by 56 cm. Electric fields were measured with an oscilloscope each time a fish was exposed (Tectronix THS 720A, Beaverton, Oregon). The oscilloscope was used to measure voltage amplitude, pulse frequency, and pulse width; and all electrical measurements of experimental treatments were within 3% of the expected value. The voltage amplitude was used for determining the voltage gradient. All electroshocking experiments were conducted at 24-26°C and 98-102 µS/cm ambient conductivity; water conductivity was adjusted by adding artificial sea salts (Instant Ocean Synthetic Sea Salt, Mentor, Ohio). Prior to experiments, homogeneity of the electric field in the plastic troughs and the glass aquarium was tested with the oscilloscope connected to a sampling electrode (Henry et al., in press), and voltage gradients were found to be uniform throughout the exposure chambers.

Laboratory experiments.-Water temperature for small fish (4-60 mm TL) held in aquaria was adjusted to 25°C with aquarium heaters for 2-3 h prior to stocking fish. Fish were taken from aquaria and stocked into troughs 20 min before electroshocking. The plastic troughs had flow-through water exchange (well water) and plastic covers. During stocking, each fish was examined and any fish with apparent deformity, injury, or abnormal swimming were replaced. Recently hatched yolk-sac larvae (2 d after hatching for channel catfish and 3 d for bluegill and largemouth bass) were stocked 10-50 fish/trough, and fish aged 5-100 d after hatching were stocked 1 fish/trough. Larger channel catfish (223-370 mm TL, 15 month old) and largemouth bass (176-300 mm TL, 13 month old) were stocked, 1 fish at a time, into the 40-L electroshocking aquarium 5 min prior to exposure.

Fish were exposed only once to a specific electric treatment of 60-Hz square-pulsed (3 ms) DC at voltage gradients of 2-16 V/cm (peak, square pulse). Each treatment consisted of 3 replicate troughs for larvae ≤ 3-d old and 10-40 fish (exposed individually) in each age group tested for fish older than 5 d. Fish in troughs or the electroshocking aquarium were randomly selected to receive 20 s of electroshock or to serve as controls. Control fish had the aluminum plate electrodes in the water to simulate shocking, but plates were not energized. For larvae ≤ 3-d old, lack of swimming was used to determine mortality and fish were observed every 8 h for up to 24 h. Fish older than 5 d were considered dead if recovery of opercular movement did not occur within 1 h. After electroshocking, fish in the glass aquarium (176-300 mm TL largemouth bass and 223-370 mm TL channel catfish) were moved to a recovery aquarium (60 L, 1 fish/aquarium) with flow-through reservoir water (described above) and observed for 1 h.

After the 1-h observation period for fish ≥ 5-d old, total length was measured, and small fish (4-60 mm TL) were preserved in 10% neutral buffered formalin or Bouin's fixative. Developmental stages of preserved fish were determined by examining fish with a dissecting microscope. Criteria for stages were based on Kendall et al. (1984). The juvenile period for channel catfish began upon depletion of the yolk (Jones et al. 1978). For other species, fish were considered juveniles upon acquisition of the full complement of fin rays. Juveniles had a body form approximating that of the adult.

Delayed (5 d) mortality was evaluated only for channel catfish; 118 fish (age 51 d, 32-39 mm TL) were individually exposed to 4 V/cm 60-Hz PDC (as described above). After 1 h, survivors were moved to 40-L glass aquaria with flow-through well water (as described above), and mortality was recorded daily for 5 d. At the same time, control fish were exposed to the same handling as electroshocked fish and were also moved to 40-L aquaria for observation for 5 d.

Field experiments.-Field validation experiments were conducted in a pond (alkalinity, 25 mg/L as $CaCO_3$; hardness, 28 mg/L as $CaCO_3$; dissolved oxygen, 7.5 mg/L; ambient conductivity, 77 µS/cm; temperature, 28.7°C) located on the Auburn University Fisheries Research Station. An electrofishing boat was positioned in the pond with the anode dropper cables (1.0 m) suspended in water 1.0-1.3 m deep. Laboratory-reared largemouth bass (age, 25 d; 14-20 mm TL) were confined in cylindrical cages (0.3 m diameter, 0.7 m height) constructed of nylon mesh and plastic pipe. Cages were anchored at specific locations near the anode dropper cables. Fish were exposed for 20 s to 60-Hz PDC generated by a Smith-Root (Vancouver, Washington) GPP electrofisher operated at 1000 V, 6.2 amps, and 80% of full pulse width. During exposure, a sampling probe (Henry et al., in press) was used to measure the voltage gradient at one point near the center of the cage, and the cage was located at different locations relative to the anode droppers to produce different voltage gradients within the cage. Three groups of 8-10 fish were exposed, and each group was exposed at a different location relative to the anode droppers (i.e., different voltage gradient). Control fish were handled the same as electroshocked fish except they were not exposed to an electric field. Fish were left in the cage, and fish that had not recovered opercular movement after 1 h were considered dead.

One day after the field study, fish from the same source as used in the pond experiment were electroshocked (60-Hz PDC) in plastic troughs (as described above) containing water from the pond (same water chemistry and temperature). Control fish were also transferred to the plastic troughs with pond water, but were not exposed to an electric field.

Statistics.-Statistical analyses were conducted with Statistical Analysis Software (SAS version 8.2; SAS Institute, Cary, North Carolina). For yolk-sac larvae ≤ 3-d old (3 replicate troughs, 10-50 fish/trough), mortality of electroshocked fish was compared to mortality of controls by analysis of variance. For older fish (exposed individually), we tested whether a quadratic function described changes in mortality rates, and used logistic regression to model fish mortality as a function of fish age or (in a separate model) as a function of fish total length. Separate models were generated for each species and voltage level. The model was

$$(1)\qquad \text{logit}\ (p) = a + b_1(A) + b_2(A^2)$$

where logit (p) is the logistic probability of a fish dying; a is intercept value; b_1 and b_2 are parameter estimates; and A is either fish age (d) or total length (mm). A model was considered significant if the p-value of the Wald chi-square statistic was <0.05.

The estimate of logit (p) was used to obtain the predicted probability of fish mortality (p):

$$(2)\qquad p = e^{\text{logit}\ (p)} \cdot (1 + e^{\text{logit}\ (p)})^{-1}$$

We used an index of rank correlation (*c*) to assess the predictive ability of the model (SAS Version 8.2, SAS Institute, Cary, North Carolina). The value of c indicates the percent effectiveness of the model for predicting a binary response. The most susceptible fish age (or TL) was calculated by setting the derivative of equation (1) equal to zero and solving for age (or TL) in each quadratic equation.

We used a *G*-test to compare mortality of fish in the field validation experiment (Zar 1984). The results for fish in the pond were combined for 2 cages that had voltage gradients that only differed by 1 V/cm. A significance level of 0.05 was used to detect differences in fish mortality when fish were exposed to homogeneous electric fields in the laboratory or to heterogeneous electric fields in cages around an electrofishing boat.

Results

After electroshocking, all species and ages of fish were immobilized. All 2-d-old channel catfish began swimming within 2 min after electroshocking, but for 3-d-old bluegill and largemouth bass, observation up to 24 h after exposure was required to determine if each larva was alive. For fish older than 3 d and that survived electroshocking, recovery of opercular movement and swimming ability generally occurred within 5 min, and all of the fish that died did not recover opercular movements at any time during the 1-h observation after the electroshock. The were no deaths of control fish aged ≥5 d.

The youngest largemouth bass that were electroshocked were 3-d-old yolk-sac larvae, and for this age there was no difference ($P = 0.65$) in mortality among electroshocked fish and control fish. Mortality of 3-d-old largemouth bass yolk-sac larvae was 13% for 2 V/cm, 17% for 4 V/cm, 9% for 8 V/cm, and 15% for controls. The yolk sac of largemouth bass was not visible by day 12, and 40% of these fish died after exposure to 4 V/cm (Figure IV-1a). Electroshocking with 4 V/cm caused high mortalities throughout the late larval and early juvenile period until day 81, when no largemouth bass were killed by 4 V/cm. Electroshocking with 2 V/cm caused lower mortality and killed fish during a narrower range of developmental stages. There were no deaths of 13–month–old largemouth bass exposed to 8 V/cm (data not shown).

Bluegill development and mortality (Figure IV-1b) were generally similar to those of largemouth bass, except that higher voltage gradients were required to obtain similar mortalities of bluegill. The youngest (3 d) and oldest (44 d) bluegill that were electroshocked with 8 V/cm had lower mortalities than the intermediate ages. None of the 3-d-old yolk-sac larvae died even after exposure to 16 V/cm. After exposure to 4 V/cm, mortalities were low for all ages except for newly transformed juveniles.

Channel catfish developed directly from yolk-sac larvae to juveniles. The youngest channel catfish (2 d) electroshocked did not die (Figure IV-1c), even at 16 V/cm. Older yolk-sac larvae and young juveniles exposed to 4 or 8 V/cm generally had high mortalities until day 91, when few fish died. There were no deaths of 15–month–old channel catfish exposed to 8 V/cm (data not shown).

The youngest Nile tilapia electroshocked were 16-d-old transitioning larvae; yolk was not present, all fin rays were present in all fins except pelvic fins, and no scales were evident. Although the voltage gradient used for all ages of Nile tilapia (16 V/cm) was higher than for any of the other species, the highest mortality was less than 30% (Figure IV-1d). The lowest mortalities for Nile tilapia were for the youngest and oldest age groups tested.

For each voltage gradient-species combination, a separate model of fish mortality was generated for both fish age and total length. All models were significant (Wald chi-square, $P < 0.05$) except for largemouth bass exposed at 2 V/cm (Wald chi-square = 3.98, $P = 0.14$) and Nile tilapia exposed at 16 V/cm (Wald chi-square = 4.18, $P = 0.12$). The index of rank correlation (c), which indicates the percent effectiveness of the model for predicting a binary response, was >0.65 for all models (Table IV-1). The age with the highest predicted mortality was 42-43 d for channel catfish, 38 d for Nile tilapia, 36-41 d for largemouth bass, and 22 d for bluegill. The total length of fish with the highest predicted mortality was 28-30 mm TL for channel catfish, 21 mm TL for Nile tilapia, 29-32 mm TL for largemouth bass, and 19-21 mm TL for bluegill.

In the experiment to evaluate delayed mortality, 80 of the 118 electroshocked channel catfish did not recover opercular movement and were considered dead 1 h after electroshocking. None of the remaining 38 fish died during the 5-d observation period. No unshocked control fish died during the 5-d observation period.

Mortalities did not differ ($G = 0.82$, $P > 0.05$) among largemouth bass exposed to similar voltage gradients generated by an electrofishing boat or in a homogeneous electric field in the laboratory (Figure IV-2). Higher voltage gradients caused higher mortalities in both situations, and all fish died after exposure to the highest voltage gradient. Exposure duration, ambient conductivity, temperature of the water, and fish size were similar for the field and laboratory exposures.

Discussion

For the four species electroshocked at different ages, an increase in electroshocking-induced mortality occurred during late larval development or soon after metamorphosis to the juvenile stage. Models of fish mortality predicted that all of these species were most susceptible soon after becoming juveniles. For largemouth bass and bluegill, the most sensitive developmental stage was predicted to be before formation of scales was complete. Channel catfish were slightly older than largemouth bass and bluegill when they were predicted to be most susceptible, and for Nile tilapia, the formation of scales was completed or nearly completed at the age of highest predicted mortality. The ontogenies of these species differ, and developmental differences among species could explain the relative differences we observed in the period of highest susceptibility.

Fish that died following electroshocking did not recover opercular movement at any time. No delayed mortality occurred for channel catfish observed for 5 d after exposure, even though 68% had died during the first hour after electroshocking. Previous studies have reported low rates of delayed mortality (<10%) for other species (Hudy 1985; Schneider 1992; McMichael 1993; Habera et al. 1996; Barrett and Grossman 1988; Ruppert and Muth 1997; Cooke et al. 1998); although previous studies have not considered the developmental stages we found most sensitive to the lethal effects of electroshocking.

Hypoxia is a potential cause of death when opercular movement is stopped following electroshocking. If hypoxia is the cause of immediate mortality after electroshocking, then the age-related differences in susceptibility we observed could be a reflection of developmentally related changes in tolerance for oxygen depravation and the capacity to obtain sufficient oxygen via cutaneous uptake. Oxygen uptake by fish is primarily cutaneous in embryos and young larvae, and branchial uptake becomes more important near the end of the larval period (Rombough 1988). For older fish, which depend on opercular movement to supply oxygen-rich water to the gills, a greater tolerance for insufficient oxygen uptake would improve chances for survival after cessation of opercular movement. Shepard (1955) found that the survival time of brook trout (*Salvelinus fontinalis*) was longer for larger fish after a sudden exposure to water with a lethal concentration of dissolved oxygen. This suggests that after fish become dependent on branchial uptake of oxygen, cessation of opercular movements is more likely to be lethal to smaller fish.

Although a period of high susceptibility to the lethal effects of electroshocking occurred for each species, the relative susceptibility appeared to differ among species. Some largemouth bass in the sensitive stages died after exposure to 2 V/cm, while 16 V/cm were required to kill Nile tilapia. Studies comparing the relative susceptibility of these species is a topic for further research.

Electroshock-induced mortality of fish has been evaluated in field studies (Pratt 1955; Bardygula-Nonn 1995; Habera et al. 1996) and in laboratory studies with homogeneous electric fields (Collins et al. 1954; Whaley et al. 1978). The voltage gradients generated around electrofishing electrodes in field situations are heterogeneous, i.e., intensity varies with the distance from an electrode (Kolz 1993). Determination of the voltage gradient and duration of exposure during electrofishing is difficult because of the high variation in electric fields (Henry et al., in press). Results of most field studies can not be compared because voltage gradient and exposure duration, which are critical factors affecting electroshocking-induced mortality (Collins et al. 1954; Lamarque 1990), are unknown for most of these studies. In laboratory tanks, the effects of homogeneous electric fields can be determined, but the applicability of these results to electrofishing in field situations with heterogeneous fields has not been evaluated previously.

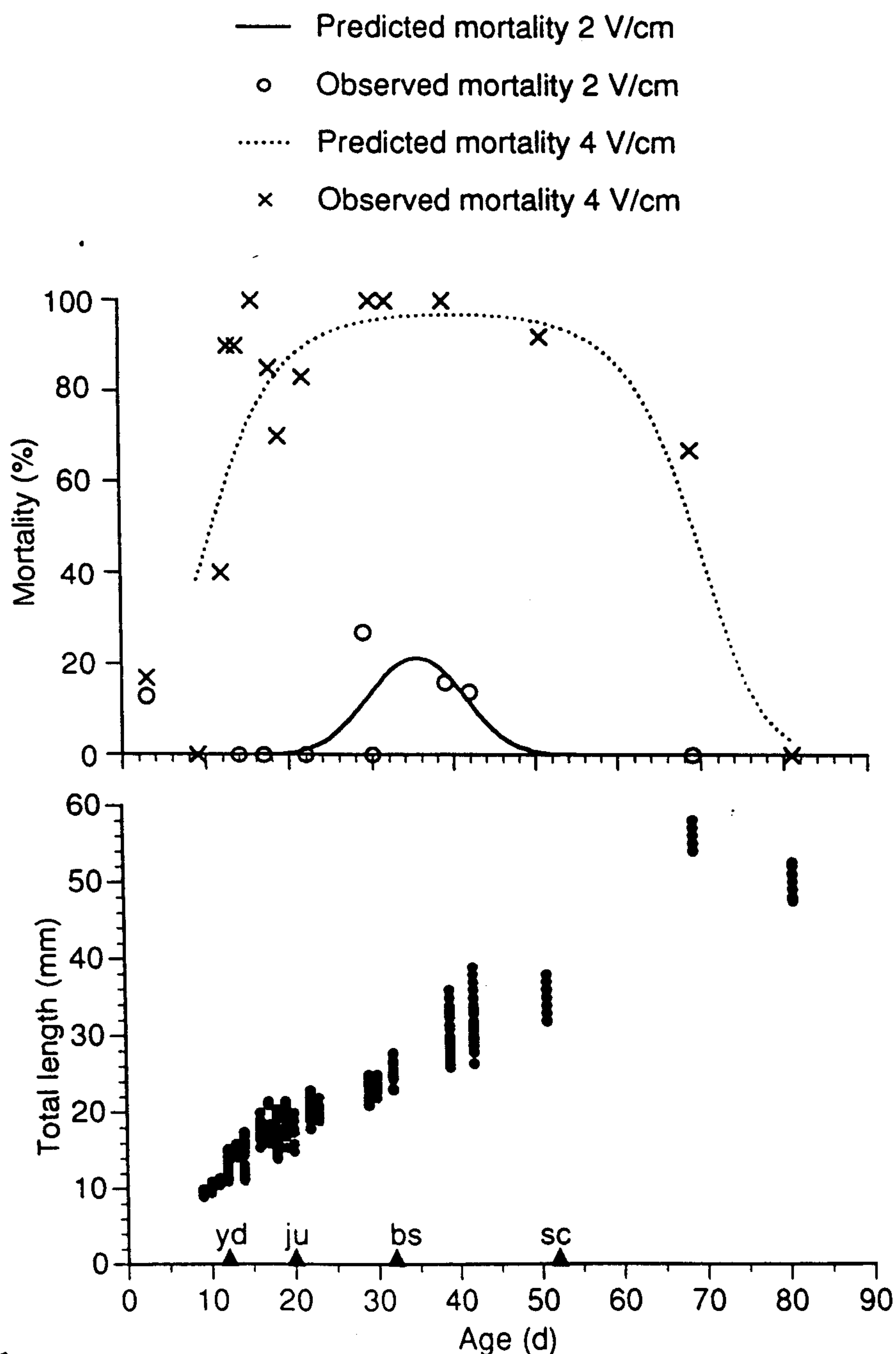
Mortalities of largemouth bass were similar in our experiment comparing heterogeneous and homogeneous electric fields. Voltage gradients were measured near the center of the cages during exposure of fish to the electric field produced by an electrofishing boat, but the minimum and maximum voltage gradients were not determined in these cages. However, confinement to a cage that was small relative to the overall size of the electric field reduced the voltage gradient range during our field experiment. We measured only the vertical and one horizontal component of the heterogeneous fields, which vary in three dimensions, but by aligning the probe with the apparent maximum voltage gradient in the horizontal plane, the error resulting from our two-dimensional measurement was probably small compared to the variation expected within the cage (Kolz 1993). Although we found that laboratory experiments with homogeneous fields were useful for determining the lethal effects of electrofishing, additional validation of our results are needed.

In the present study, mortality of electroshocked fish varied in relation to fish developmental stage; however, the significance of this result to the practice of electrofishing will require additional evaluation. The importance of elevated mortality of young fish on fish populations may be negligible for some species or even beneficial to reduce excess recruitment [e.g., centrarchid populations (Spencer 1967b)], or viewed as highly detrimental for endangered species (Nielsen 1998).

Table IV-1. Predicted age and total length of channel catfish, bluegill, largemouth bass, and Nile tilapia that were most susceptible to electroshocking-induced mortality. For each model, the index of rank correlation (*c*), which indicates the percent effectiveness of the model for predicting a binary response, is in parenthesis.

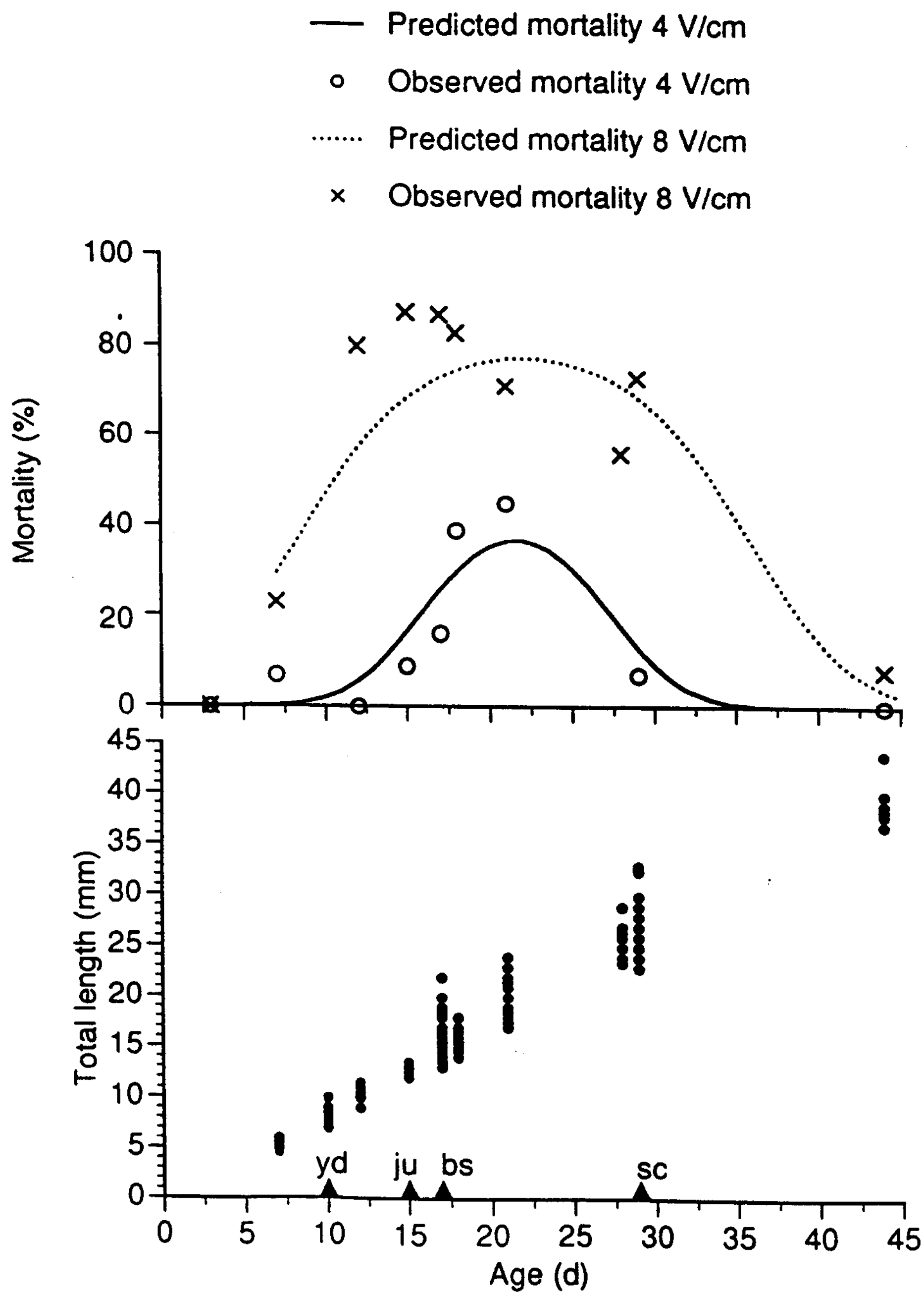
Species	Voltage gradient (V/cm)	Age (d) of fish when most susceptible	Total length (mm) of fish when most susceptible
Bluegill	4	21.6 (0.78)	19.3 (0.72)
Bluegill	8	22.1 (0.74)	20.8 (0.73)
Largemouth bass	2	36.0 (0.66)	29.3 (0.77)
Largemouth bass	4	41.0 (0.85)	32.1 (0.82)
Channel catfish	4	42.7 (0.75)	30.3 (0.76)
Channel catfish	8	42.0 (0.81)	27.5 (0.81)
Nile tilapia	16	37.7 (0.69)	21.0 (0.74)

Figure IV 1. Relation of electroshocking-induced mortality with fish age (days after hatching), total length, and developmental stage for (a) largemouth bass, (b) bluegill, (c) channel catfish, and (d) Nile tilapia. On the age-axis, labeled points indicate stages and developmental events: yd, yolk sac depleted; ju, juvenile (fin rays complete); bs, beginning of scale formation; sc, scale formation complete or nearly so. No control fish died except for 3-d-old largemouth bass yolk-sac larvae which had 15% mortality.



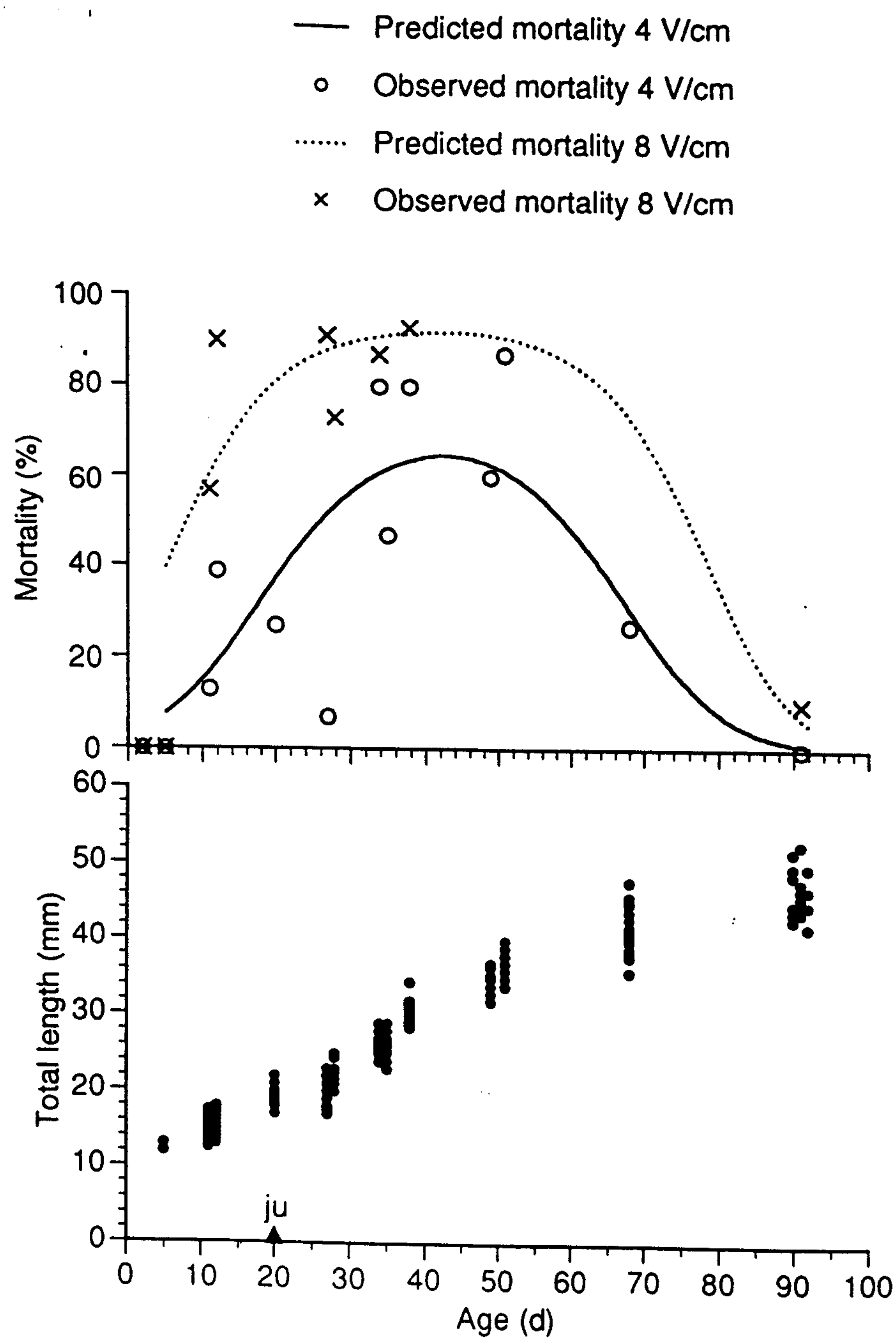
Predicted mortality 2 V/cm
Observed mortality 2 V/cm
Predicted mortality 4 V/cm
Observed mortality 4 V/cm
Mortality (%)
100
80
60
40
20
0
Total length (mm)
60
50
40
30
20
10
0
yd
ju
bs
sc
0
10
20
30
40
50
60
70
80
90
Age (d)

Figure IV-1a.



Predicted mortality 4 V/cm
Observed mortality 4 V/cm
Predicted mortality 8 V/cm
Observed mortality 8 V/cm
Mortality (%)
100
80
60
40
20
0
Total length (mm)
45
40
35
30
25
20
15
10
5
0
yd
ju
bs
sc
0
5
10
15
20
25
30
35
40
45
Age (d)

Figure IV-1b.



Predicted mortality 4 V/cm
Observed mortality 4 V/cm
Predicted mortality 8 V/cm
Observed mortality 8 V/cm
Mortality (%)
100
80
60
40
20
0
Total length (mm)
60
50
40
30
20
10
0
ju
0
10
20
30
40
50
60
70
80
90
100
Age (d)

Figure IV-1c.

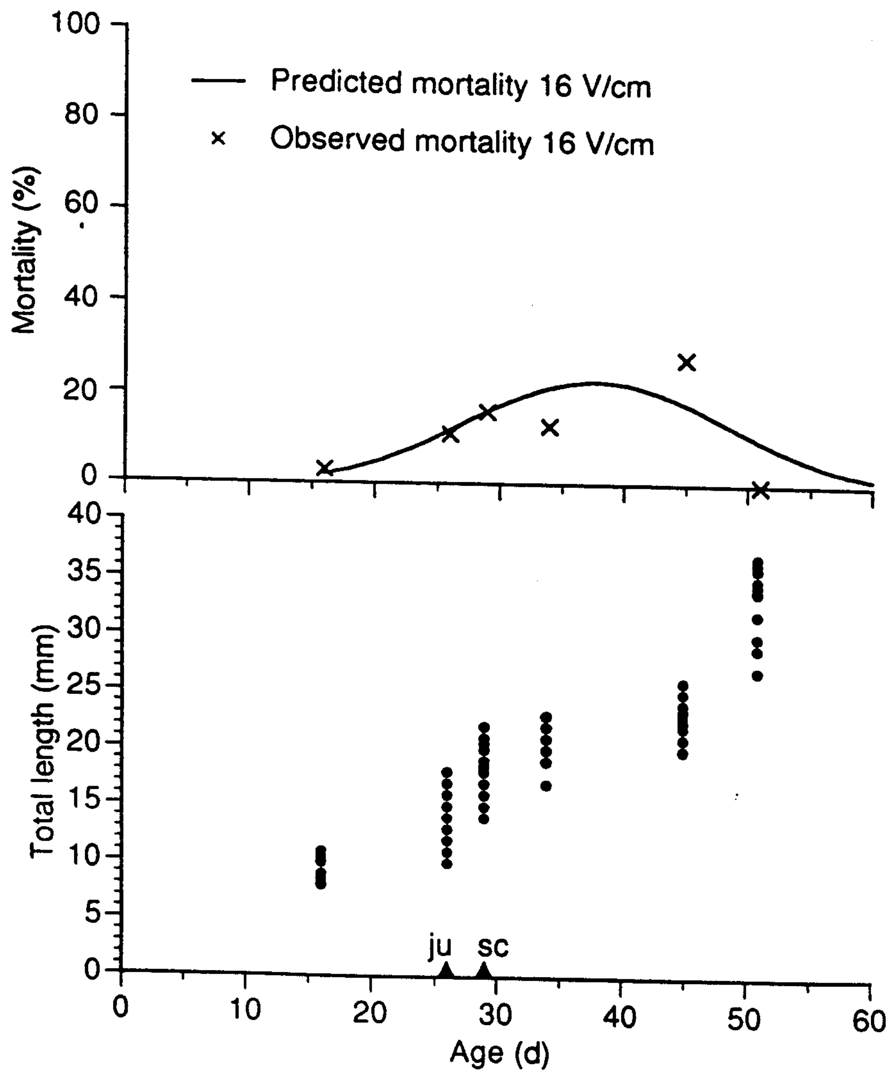

100
80
60
40
20
0
Mortality (%)
Predicted mortality 16 V/cm
Observed mortality 16 V/cm
40
35
30
25
20
15
10
5
0
Total length (mm)
ju
sc
0
10
20
30
40
50
60
Age (d)

Figure IV-1d.

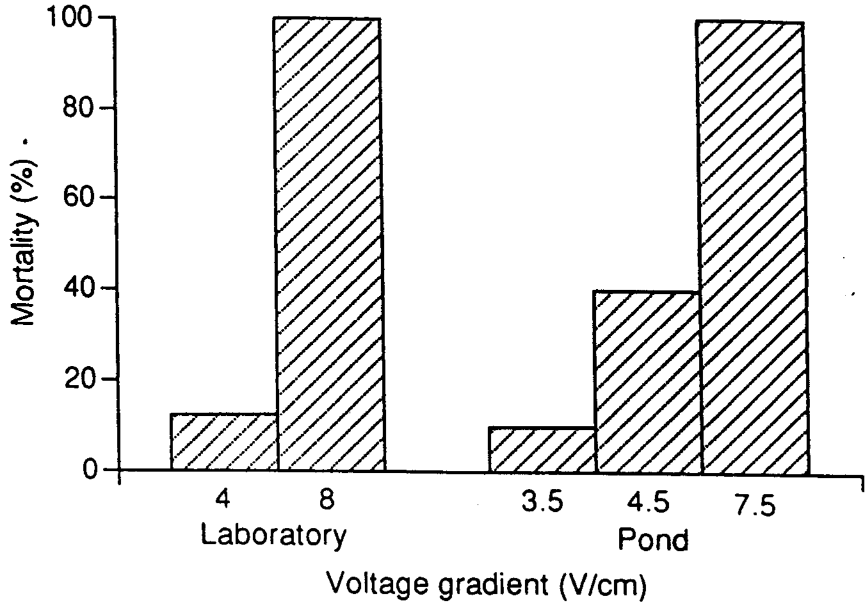


Figure IV-2. Field validation experiment with largemouth bass (age 25 d; 14-22 mm TL). Bars indicate the mortality of 9-12 fish. For the pond experiment, an electrofishing boat was used to shock fish held in cages. For the laboratory experiment, fish in plastic troughs were exposed to a homogeneous electric field. Fish in both pond and laboratory experiments were exposed (20 s) to 60-Hz PDC in water of 76-77 μS/cm ambient conductivity.

V. SUSCEPTIBILITY OF TEN FISH SPECIES TO ELECTROSHOCKING-INDUCED MORTALITY AND SELECTION OF ELECTRIC CURRENTS TO REDUCE MORTALITY

Abstract.-We exposed 10 fish species to homogeneous electric fields of 60-Hz pulsed DC (PDC) at voltage gradients of 0.5 to 16 V/cm in water of 100 µS/cm ambient conductivity. All fish were newly transformed juveniles, except blackbanded darters *Percina nigrofasciata* were juveniles and adults and western mosquitofish *Gambusia affinis* were adults. Blackbanded darters had the highest immediate (1 h) mortality, while paddlefish *Polyodon spathula*, Nile tilapia *Oreochromis niloticus*, and western mosquitofish were least susceptible. Paddlefish and western mosquitofish did not die after exposure to any electric fields, but for all other species mortality increased with voltage gradient. Other species tested were: largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, black crappie *Pomoxis nigromaculatus*, striped bass *Morone saxatilis*, channel catfish *Ictalurus punctatus*, and rainbow trout *Oncorhynchus mykiss*. Largemouth bass, bluegill, black crappie, and channel catfish were also exposed to DC, PDC from 7.5 to 120 Hz, and to a complex pulse system (CPS, consisting of 3 pulses of 240-Hz repeated at 15 Hz) to determine the types of electric currents most likely to kill fish. The effects of pulse width and exposure time were also investigated. The 7.5-Hz PDC resulted in the lowest mortality, followed by

DC and 15-Hz PDC. Highest mortality was caused by 60- and 120-Hz PDC, which killed all fish at 16 V/cm. Increasing pulse width or exposure time also increased mortality. Fish mortality can be reduced by avoiding habitats where sensitive species are found and by selecting less injurious electric currents during electrofishing.

Introduction

Fish can be negatively affected by the electric currents used for electrofishing (Hauck 1949; Collins et al. 1954; Reynolds 1996), but little information is available about the types of electric currents that are likely to kill fish or about which fish species are most vulnerable. Additional information on these subjects is important so that electrofishing procedures can be modified to reduce injury of sensitive species.

While fish have been reported to die following electroshocking (Snyder 1992), some studies have concluded that fish mortality is negligible (Whaley et al. 1978; Hudy 1985; Schneider 1992), and other studies report that fish mortality is important (Collins et al. 1954; Nielsen 1998). Unfortunately, most studies reporting fish mortality following electroshocking are not comparable because of differences in electric field characteristics and experimental design. Studies of fish mortality in field electrofishing situations are difficult to compare because the high variation of in-water voltage gradients around electrodes (Kolz 1993) prevents an accurate determination of the exposure duration and electric field characteristics to which fish are exposed. Homogeneous electric fields with defined electrical characteristics have been used to evaluate electroshocking effects on fish (Collins et al. 1954), but except for a comparison of two species by Whaley et al.

(1978), multiple-species comparisons of mortality after exposure to homogeneous fields have not been conducted. Laboratory experiments with homogeneous electric fields can be related to field electrofishing conditions (Chapter IV), and are appropriate for multiple species comparisons of electrofishing-induced mortality.

The usefulness of previous multiple-species studies of electroshocking-induced mortality is limited because of inadequate controls, unknown voltage gradient or exposure duration, and low mortality (Pratt 1955; Hudy 1985; Schneider 1992; Bardygula-Nonn et al. 1995). One potential explanation for the low fish mortality reported in these studies is that particularly sensitive species and life stages were not evaluated.

The stage of development is related to mortality in some fish species (Chapter IV); therefore, differences in life history stage should be considered in comparisons of electrofishing-induced mortality among species. Mortality was higher for newly transformed juveniles than for younger or older fish following electroshocking of four species (Chapter IV). Studies of electrofishing-induced mortality of larger fish are conflicting: some conclude higher mortality for smaller fish (Habera et al. 1996) while others have found no difference in mortality with respect to fish size (Collins et al. 1954; Pratt 1955; Whaley et al. 1978). No studies have reported higher mortality for larger fish; however, spinal injury can increase with fish size (Hollender and Carline 1994; Thompson et al. 1997a), although there does not appear to be a connection between spinal injury and mortality (Spencer 1967a; Hudy 1985; Habera et al. 1996).

Fish mortality following electroshocking has not been determined for any species over a wide range of DC and pulsed DC (PDC) waveforms. Two studies (Collins et al. 1954; Whaley et al. 1978) found that fish mortality increased with pulse frequency; however, the frequencies evaluated (1.6- to 16-Hz PDC) were lower than frequencies commonly used in electrofishing operations at present (Reynolds 1996). In studies of DC and 30- to 120-Hz PDC, which are currently used for electrofishing, fish mortality was negligible, and differences in fish mortality among waveforms were not detected (McMichael 1993; Bardygula-Nonn et al. 1995). Results of Cooke et al. (1998) suggest that initial mortality (fish did not recover) of greenside darters *Etheostoma blenniodes* was higher for 80-Hz PDC than 60-Hz PDC; however, only 9% (4 fish) of the fish died at 80 Hz and accurate assessment of the instream exposure duration and intensity was not possible. Although previous studies have not found significant differences in mortality among current types, studies have not considered fish smaller than 45 mm total length (TL), which can be most susceptible to electroshocking-induced mortality (Chapter IV).

The first objective of the present study was to compare immediate (1 h) mortality of 10 species of fish following a 20-s exposure to 60-Hz PDC. The ten species were largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, black crappie *Pomoxis nigromaculatus*, blackbanded darter *Percina nigrofasciata*, striped bass *Morone saxatilis*, Nile tilapia *Oreochromis niloticus*, channel catfish *Ictalurus punctatus*, rainbow trout *Oncorhynchus mykiss*, western mosquitofish *Gambusia affinis*, and paddlefish *Polyodon spathula*. The second objective was to evaluate electric field characteristics on mortality of largemouth bass, bluegill, black crappie, and channel catfish. Fish were exposed to DC, 7.5- to 120-Hz PDC at pulse widths of 1 to 5 ms, or a complex pulse system (CPS; composed of 3 pulses of 240 Hz repeated at 15 Hz) at voltage gradients of 2-16 V/cm. We also evaluated the effect of exposure durations of 5 to 20 s on mortality. In all experiments, all fish were newly transformed juveniles except blackbanded darters which were juveniles and adults and western mosquitofish which were adults.

Methods

Experimental animals.-Fish were obtained from the Auburn University Fisheries Research Station, streams located in Lee County, Alabama, or from fish hatcheries (Table V-1). After collection from streams, blackbanded darters were kept for 1-2 weeks in 80-L laboratory aquaria (20-25 fish/aquarium; 10% water change every 2 d) with pond water. This water had the following characteristics: alkalinity, 34 mg/L as $CaCO_3$; hardness, 30 mg/L as $CaCO_3$; pH, 7.2; dissolved oxygen, 7.0 mg/L; conductivity, 99 µS/cm; and temperature, 24-26°C. After field collection, western mosquitofish were kept 1-2 weeks in 40-L flow-through (0.25 L/min) aquaria with well water. All other fish were kept in 40-L aquaria with flow-through well water of the following characteristics: alkalinity, 25 mg/L as $CaCO_3$; hardness, 23 mg/L as $CaCO_3$; pH, 7.1; dissolved oxygen, > 6.0 mg/L; conductivity, 80 µS/cm; and temperature, 18°C. Fish were fed zooplankton, brine shrimp (*Artemia*), and powdered fish feed. In addition, western mosquitofish and blackbanded darters were fed frozen brine shrimp and blood worms (*Chironomus*).

Electric fields.-Electric fields were generated by an electrofishing pulse box (Coffelt, TEG-10 Proto 1, Flagstaff, Arizona) modified to be powered by standard 110 V AC and to produce DC and square-pulsed DC electric fields. Electricity was delivered to the water of exposure chambers (plastic troughs, 38.1 X 7.7 cm, and 5.1 cm deep) with aluminum plate electrodes, which conformed to the cross-sectional area of the troughs. Electrodes were separated by 30 cm in troughs and were removable. Electric fields were measured with an oscilloscope each time a fish was exposed (Tectronix THS 720A, Beaverton, Oregon). The oscilloscope was used to measure voltage amplitude, pulse frequency, and pulse width; and all measurements of experimental treatments were within 3% of the expected value. Prior to experiments, homogeneity of the electric field in the plastic troughs and the glass aquarium was tested with the oscilloscope connected to a sampling electrode (Henry et al., in press), and voltage gradients were found to be uniform throughout the exposure chambers.

Species comparison.-Electroshocking experiments were conducted with 10 species of fish (Table V-1). The plastic troughs used to expose fish to electroshocking had flow-through water (well water) and plastic covers. For all fish except rainbow trout, prior to stocking troughs the water temperature in the holding aquaria was adjusted to 25°C with aquarium heaters for 2-3 h. Fish were stocked into troughs (1 fish/trough) 20 min before electroshocking. During stocking, each fish was examined, and fish with apparent deformity, injury, or abnormal behavior were replaced. Electroshocking of each fish species was conducted at 24-26°C (17.5-18.5°C for rainbow trout) and 98-102 μS/cm; water conductivity was adjusted by adding artificial sea salts (Instant Ocean Synthetic Sea Salt, Mentor, Ohio).

Fish in plastic troughs were randomly selected to receive an electric shock or to serve as controls. Control fish were treated the same as fish that received an electric shock except the electrodes were not energized. Electric treatments and the controls were replicated 5-40 times (1 fish/replicate; Table V-1). Treatment fish were exposed to 60-Hz square pulsed (3 ms) DC for 20 s, and immediate mortality was evaluated for all fish in all experiments. After treatment, fish were left in the trough where they were shocked, the electrodes were removed within 1 min, and water flow was restored to the trough. Fish were observed during exposure, to determine if they were immobilized, and after exposure to observe recovery of opercular movement and swimming ability. Fish were considered dead if no opercular movement was observed within 1 h after exposure to electricity. After determination of mortality, fish total length was measured.

Comparison of electric currents.-Experimental procedures and handling of fish were the same as described above. Largemouth bass, bluegill, black crappie, and channel catfish were tested in separate experiments, and after stocking, fish were randomly selected to receive an electric treatment or to serve as controls. Treatment fish were exposed to an electric field for 20 s, unless exposure time was used as a treatment variable. Electric treatments were DC, 7.5-, 15-, 30-, 60-, and 120-Hz pulsed (3 ms) DC, or CPS for each species except for largemouth bass which were exposed to all electric field types except 7.5 and 15 Hz. The electric field intensity was from 2-16 V/cm. The effect of pulse width on mortality was evaluated for 1 and 4 ms at pulse frequencies of 7.5 to 120 Hz (4 and 8 V/cm) for channel catfish, and for largemouth bass at 1 and 5 ms at 30 to 120 Hz (4 V/cm). The relation of exposure duration on mortality was evaluated after 5 to 20 s durations of 60-Hz PDC for largemouth bass and channel catfish at 4 V/cm and bluegill at 8 V/cm. Determination of mortality was as described above.

Statistics.-The effects of species, voltage, current type, and pulse width were assessed in the following model of fish mortality (π):

$$\log (\pi_{ijk}/1- \pi_{ijk}) = \mu + \alpha_h + \beta_i + \gamma_j + \delta_k ,$$

where $\log (\pi_{ijk}/1- \pi_{ijk})$ was the log likelihood of a fish dying, μ was the intercept value, α_h was the fixed effect of species h, β_i was the fixed effect of voltage i, γ_j was the fixed effect of current type j, and δ_k was the fixed effect of pulse width k. The empirical logistic transformation, which adds 0.5 to the number of fish dying and 1 to the number at risk for each treatment combination, was used to transform mortality data (Cox 1970). Model estimation was performed using iterative maximization of the likelihood function (PROC GENMOD; SAS version 8.0; SAS institute, Inc., Cary, North Carolina). Independent variables and their interactions were included in the final model (Table V-2) if they were significant ($P < 0.05$) based on the likelihood ratio test (Pedhazur 1997). The overall goodness of fit of the model was based on the scaled deviance of the fitted model. Differences among species, current types, or pulse widths were tested by pairwise comparisons (ESTIMATE statement in PROC GENMOD) and considered significant if a $P < 0.05$ was obtained for the Wald χ^2 statistic. The relation of exposure time on mortality was evaluated by logistic regression at a probability level of $P < 0.05$ (PROC LOGISTIC in SAS).

Results

Species comparison.-All fish were immobilized within 5 s by all voltage gradients except for paddlefish, Nile tilapia, and western mosquitofish, which were immobilized within 5 s only after exposure to 16 V/cm. Immobilization of these species at voltage gradients less than 16 V/cm required exposure durations of more than 10 s. Following immobilization, fish that survived shocking generally restored opercular movement and recovered swimming ability within 5 min after shocking. Paddlefish appeared to recover most rapidly and remained immobilized <30 s while other species generally required longer recovery times. Blackbanded darters and largemouth bass required more time to recover than other species and often remained immobilized for >2 min after voltage gradients of 1 or 2 V/cm. Two female western mosquitofish electroshocked at 8 V/cm had premature parturition; all offspring from 1 female survived while all offspring from the other female died.

Fish that did not recover opercular movement within 1 h were considered dead and frequently had gaping mouths, flared opercula, and arched backs. None of the control fish of any species died, and following electroshocking, no paddlefish or western mosquitofish died. For all other species, mortality increased with voltage gradient (Figure V-1). Fish species ($\chi^2 = 21.7$, df = 5, $P < 0.05$) and voltage gradient ($\chi^2 = 113.8$, df = 7, $P < 0.05$) were the only terms that contributed significantly to the model comparing fish mortality among all species (Table V-2; scaled deviance = 23, df = 15). Blackbanded darters had significantly higher mortality than all other species. The species with the next highest mortality were largemouth bass, striped bass, and channel catfish, which did not differ significantly. Mortality of channel catfish also did not differ from rainbow trout or bluegill, but was significantly higher than for black crappie. Nile tilapia had low mortality, and only 27% died after exposure to 16 V/cm.

Comparison of electric currents.-For species (largemouth bass, bluegill, black crappie, and channel catfish) exposed to different current types, all fish were immobilized during electroshocking; however, the onset of immobilization occurred after 5 s for fish exposed to 7.5 Hz at 2-4 V/cm while all other electric fields immobilized fish within 5 s. Recovery of opercular movements and swimming ability was similar to that described above. No control fish died during experiments.

The model to determine the effect of current type (Table V-2) on the probability of fish mortality was described by voltage gradient and pulse frequency (scaled deviance = 161, df = 1.88). Voltage gradient (χ^2 = 350, df = 4, $P < 0.001$) and pulse frequency (χ^2 = 110, df = 6, $P < 0.001$) contributed significantly to the model, but species (χ^2 = 5.4, df = 3, $0.25 < P < 0.1$) and interactions among independent variables did not. Fish mortality increased with voltage gradient for each current type tested (Figure V-2). In general, fish mortality increased with pulse frequency. Mortality was lowest after exposure to 7.5 Hz, and increased at both 15 Hz and 30 Hz. The highest mortality occurred after exposure to 60- and 120-Hz PDC, which did not differ significantly. Direct current resulted in higher mortality than 7.5 Hz and lower than 30 Hz, but did not differ from mortality induced by 15 Hz. Mortality after exposure to CPS was lower than 60 and 120 Hz and higher than DC, 15 Hz, and 7.5 Hz; but did not differ from 30 Hz.

The effect of pulse width on fish mortality was modeled separately for each species (Table V-2). For the experiment with channel catfish, significant terms in the model of mortality (scaled deviance = 14.5, df = 13) were pulse width (χ^2 = 5, df -= 1, $P < 0.05$), pulse frequency (χ^2 = 55, df = 4, $P < 0.001$), and voltage gradient (χ^2 = 33.8, df = 1, $P < 0.001$). Higher mortality of channel catfish occurred after exposure to 4-ms pulse widths than 1-ms pulse widths; however, at 8 V/cm of 7.5 Hz, 1 fish died at 1 ms while no fish died at 4 ms (Figure V-3). In the experiment with different pulse widths, differences in mortality of channel catfish relative to current type were similar to those determined in the model for current type (see above), except mortality after exposure to 120 Hz was significantly lower than at 60 Hz and did not differ from 30 Hz. For largemouth bass, pulse width ($\chi^2 = 5.6$, df = 1, P < 0.01) and pulse frequency ($\chi^2 = 22.5$, df = 2, $P < 0.001$) were included in the model of fish mortality (scaled deviance = 2.1, df = 2). Higher mortality of largemouth bass occurred after exposure to 5-ms pulse widths than 1-ms pulse widths, although all fish died at both pulse widths at 120 Hz (Figure V-3). Differences in mortality of largemouth bass relative to pulse frequency were similar to those determined in the model for current type (see above). Mortality of largemouth bass, bluegill, and channel catfish increased ($P < 0.05$) with exposure time (Figure V-4).

Discussion

We found that immediate mortality of electroshocked fish was species dependent. Western mosquitofish and paddlefish did not die following exposure to 16 V/cm, while 18% of blackbanded darters died after exposure to 1 V/cm. Holliman (1998) reported that some species appear resistant to capture by electrofishing, but information on the relative susceptibility of different species to electroshocking-induced mortality is lacking. Salmonids have been most commonly used in studies of the negative effects of electroshocking (Snyder 1992); however, our results indicate that rainbow trout were less sensitive than other species to the lethal effects of electroshocking.

Of the species we tested, blackbanded darters were most susceptible to electroshocking-induced mortality. The effects of electroshocking on blackbanded darters have not been previously evaluated, and comparison with studies of other darter species is difficult because of differences in methods. In a study with greenside darters *Etheostoma blenniodes*, Cooke et al. (1998) reported <10% immediate mortality for fish exposed to 60- or 80-Hz PDC during backpack electrofishing in a small stream. While differences in susceptibility could exist between blackbanded and greenside darters, the differences between our results and those of Cooke et al. (1998) could also relate to exposure duration or voltage gradient [not reported in Cooke et al. (1998)]. Whaley et al. (1978) reported that mortality of fantail darters *Etheostoma flabellare* increased with electroshock duration, and the predicted mortality after a 20-s exposure would be <20%. The pulse frequencies used by Whaley et al. (1978) were only 1.6- to 16-Hz PDC (4 V/cm, conductivity 154 μS/cm), and the higher pulse frequency used in our study could have caused the higher mortality of blackbanded darters.

Immediate mortality after electroshocking depends on developmental stage of the fish; newly transformed juveniles were the most susceptible stages for largemouth bass, bluegill, Nile tilapia, and channel catfish (Chapter IV). For species other than western mosquitofish and blackbanded darters, we used newly transformed juveniles, although the most susceptible developmental stage has not been determined for most of the species we used. If this developmental period is not the most susceptible for some of the species tested, then the relative susceptibility among species we reported can be questioned. Blackbanded darters were the most sensitive species we tested, so use of a more sensitive stage of development for this species would not have affected their susceptibility to immediate mortality relative to other species tested. Western mosquitofish were adults and no fish died following electroshocking; therefore, if younger life stages had been tested the susceptibility of western mosquitofish could rank higher relative to some other species. Fish mortality during electrofishing is often reported as low or negligible (Hudy 1985; Schneider 1992), and the relatively high mortalities for some species in our study could result from the use of more sensitive developmental stages.

For the species killed by electroshock, mortality increased with voltage gradient, which is consistent with results of a previous study of fish exposed to homogeneous electric fields (Collins et al. 1954). We selected the voltage gradients used in the present study because similar voltage gradients occur around electrofishing boats (Henry et al., in press). The voltage gradients around electrofishing boats are heterogeneous; therefore, intensity varies with measurement location relative to the electrodes (Kolz 1993). Voltage gradients of 16-20 V/cm can occur within 5 cm of anode droppers, and 2 V/cm gradients can occur near the bow of electrofishing boats (Henry et al., in press).

While voltage gradients around electrofishing equipment are heterogeneous, pulse frequency is consistent throughout the electric field, and our results with largemouth bass, bluegill, black crappie, and channel catfish indicate that pulse frequency is related to fish mortality. We found that fish mortality increased with pulse frequency, and all fish died at 60 and 120 Hz (16 V/cm), the highest pulse frequencies tested. Other researchers found that mortality increased with pulse frequency (Collins et al. 1954; Whaley et al. 1978), although the frequencies tested were below 20 Hz. In our study, 7.5 Hz resulted in the lowest mortality among all current types tested. Higher pulse frequencies have also been related to increased occurrence of vertebral injury (Sharber and Carothers 1988a; McMichael 1993).

Direct current has been considered relatively harmless to fish (Rayner 1949), and has been recommended to reduce the negative effects of electrofishing on fish (Reynolds 1996). For the newly transformed juvenile fish in this study, mortality caused by DC was lower than for all frequencies of PDC except 15 Hz, which did not differ from DC, and 7.5 Hz which killed fewer fish. While fish mortality by DC has been reported previously (Spencer 1967b), comparisons of electroshocking-induced mortality among DC and various frequencies of PDC have not been conducted. Frequency of spinal injury caused by DC can be as high as for PDC (Schill and Elle 2000). A disadvantage of DC for electrofishing is that electric power requirements are higher than for PDC, which can limit electrofishing with DC in some areas (Reynolds 1996).

The CPS waveform was developed to reduce electroshocking-induced injury caused by high frequency (≥60 Hz) PDC while maintaining sampling efficiency during electrofishing (Sharber et al. 1994). The CPS waveform is a 15-Hz PDC with each pulse divided into three pulses (240 Hz). We found that CPS caused higher mortality than 15 Hz, did not differ from 30 Hz, but killed fewer fish than 60 or 120 Hz. No mortality has been reported following electroshocking with CPS in previous studies (Sharber et al. 1994; Muth and Ruppert 1996; Ruppert and Muth 1997); however, the lack of mortality could be because fish were not examined at developmental stages highly susceptible to electroshocking-induced mortality (Chapter VI).

In all experiments, we determined the immediate mortality of fish after electroshocking, rather than delayed mortality hours or days later. Electroshocking-induced mortality of fish has been evaluated at various times after exposure, ranging from 1 h (Collins et al. 1954) to several weeks (Schneider 1992; Ainslie et al. 1998). Delayed mortality is low (<10%) in most studies (Barrett and Grossman 1988; Habera et al. 1996; Ruppert and Muth 1997; Cook et al. 1998). We also found that electroshocked channel catfish that were alive after 1 h did not die during the following 5 d, even though the immediate mortality was 68% (Chapter IV).

Fish mortality data from field electrofishing studies are difficult to interpret because the exposure duration and voltage gradient are usually unknown. In electrofishing situations, voltage gradients are heterogenous, and the position of the fish changes relative to the electrodes. While the effect of exposure duration on fish mortality has not been evaluated in heterogeneous electric fields; in homogeneous fields, longer exposure durations lead to higher fish mortality for all species tested (Collins et al. 1954; Whaley et al. 1978; present study). The ability to quantify the electroshocking exposure is an advantage of studies conducted in homogeneous electric fields, and one study has shown that when voltage gradient and duration of exposure were similar, mortality was similar for fish exposed to homogeneous fields in laboratory tanks or to heterogeneous fields produced by an electrofishing boat (Chapter IV).

It is important to recognize that all species and life stages are exposed to electric fields during electrofishing, and negative effects of electrofishing are not limited to the target fish. Exposure of endangered species to electric fields during electrofishing is a concern in some areas (Nielsen 1998), and susceptible species need to be identified so electrofishing can be avoided when highly vulnerable species are present. A wide variety of species, including some darter species, are recognized as threatened or endangered (Warren et al. 2000), and determining susceptibility of these species will likely depend on extrapolating from results of surrogate species (e.g., blackbanded darters). While our study identified species that are particularly vulnerable to the lethal effects of electroshock, fish mortality can be reduced by avoiding habitats where sensitive species and life stages exist, by reducing exposure to high voltage gradients near electrodes, reducing frequency and pulse width of PDC, and by avoiding long exposure durations.

Table V-1. Fish used in electroshocking experiments. All fish were newly transformed juveniles except blackbanded darters were older juveniles and adults, and western mosquitofish were adults.

Species	Total length (mm)	Age (d)	Source	Number/treatment
Largemouth bass	21-29	30-40	AU[a]	10-12
Bluegill	13-20	20-30	AU[a]	10-12
Black crappie	21-31	35-45	AU[a]	10-12
Blackbanded darter	30-90		AL[b]	5-13
Striped bass	23-30	40-50	GA[c]	15-30
Nile tilapia	20-30	20-30	AU[a]	30-40
Western mosquitofish	25-51		AL[d]	9-12
Channel catfish	21-30	30-47	AU[a]	10-12
Rainbow trout	24-36	25-30	TN[e]	15-30
Paddlefish	24-29	18-25	AU[a]	5-11

[a]Hatched in captivity at Auburn University Fisheries Research Station.

[b]Wild fish obtained from Uchee Creek, Chattahoochee River drainage, Lee County, Alabama.

[c]Savannah River Strain, Georgia Department of Natural Resources, McDuffie Hatchery.

[d]Wild fish from Saugahatchee Creek, Tallapoosa River drainage, Lee County, Alabama.

[e]EED strain, Tennessee Wildlife Resources Agency, Buffalo Springs Hatchery

Table V-2. Models of fish mortality (π) selected to assess the effects of species, voltage gradient, current type, and pulse width. Terms that contributed significantly (χ^2, $P < 0.05$) were included in final models, and terms that were considered for inclusion in each model are in brackets. The terms were: μ, the intercept value; α_h, the fixed effect of species h; β_i, the fixed effect of voltage i; γ_j, the fixed effect of current type j; and δ_k, the fixed effect of pulse width k. Interactions among these effects were also tested and are indicated by the Greek symbols in parentheses followed by two subscripts.

Name of model	Terms in model
Species	$\mu, \alpha_h, \beta_i,$
	$[\mu, \alpha_h, \beta_i, (\alpha\,\beta)_{hi}]$
Current type	$\mu, \beta_i, \gamma_j, (\alpha\,\gamma)_{hj}$
	$[\mu, \alpha_h, \beta_i, \gamma_j, (\alpha\,\beta)_{hi}, (\alpha\,\gamma)_{hj}, (\beta\,\gamma)_{ij}]$
Pulse width (channel catfish)	$\mu, \beta_i, \delta_k, \gamma_j$
	$[\mu, \beta_i, \gamma_j, \delta_k, (\beta\,\gamma)_{ij}, (\beta\,\delta)_{ik}, (\gamma\,\delta)_{jk},]$
Pulse width (largemouth bass)	μ, γ_j, δ_k
	$[\mu, \gamma_j, \delta_k, (\gamma\,\delta)_{jk}]$

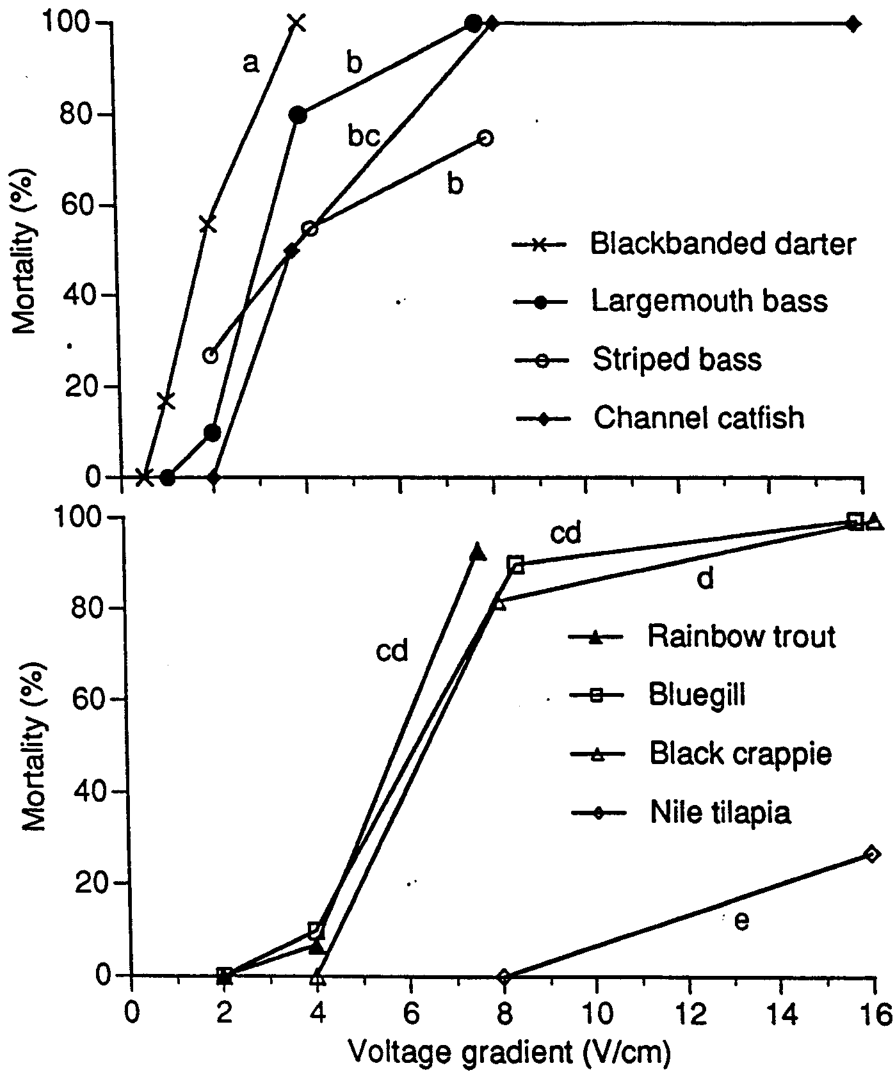


Figure V-1. Mortality of fish 1 h after a 20-s exposure to various voltage gradients of 60-Hz PDC in water with 100 μS/cm ambient conductivity. Lines with different letters are significantly different ($P < 0.05$). See Table V-1 for information about the test fish. No paddlefish or western mosquitofish died even after exposure to 16 V/cm gradients.

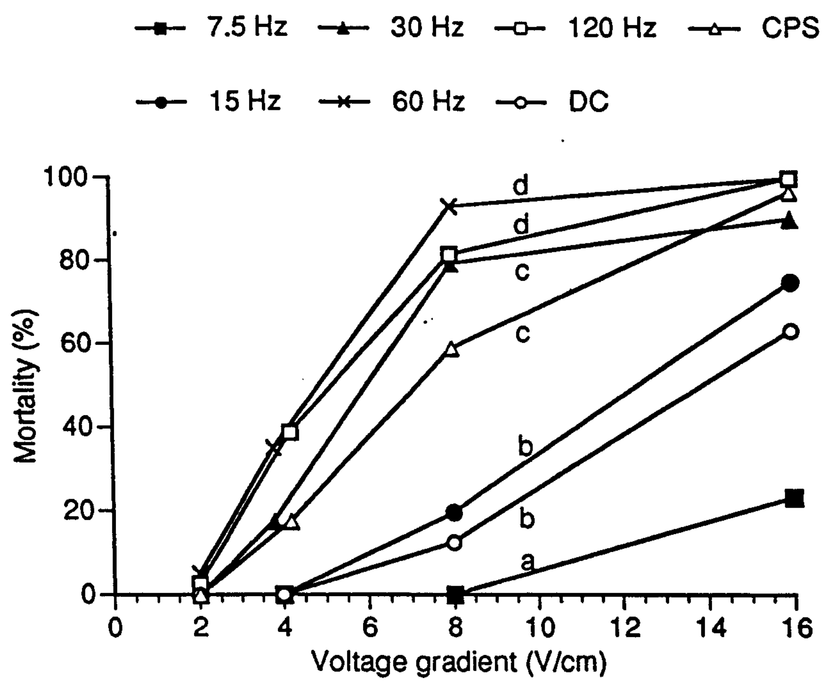


Figure V-2. Effect of electric current type on mortality of channel catfish (21-30 mm TL), black crappie (21-31 mm TL), bluegill (13-20 mm TL), and largemouth bass (22-29 mm TL) 1 h after a 20-s exposure to DC or pulsed (3 ms) DC electric fields in water with 98-102 μS/cm ambient conductivity. Fish species did not contribute significantly to models of fish mortality; therefore, the effect of current type was considered over all species combined. Lines for each current type with different letters were significantly ($P < 0.05$) different.

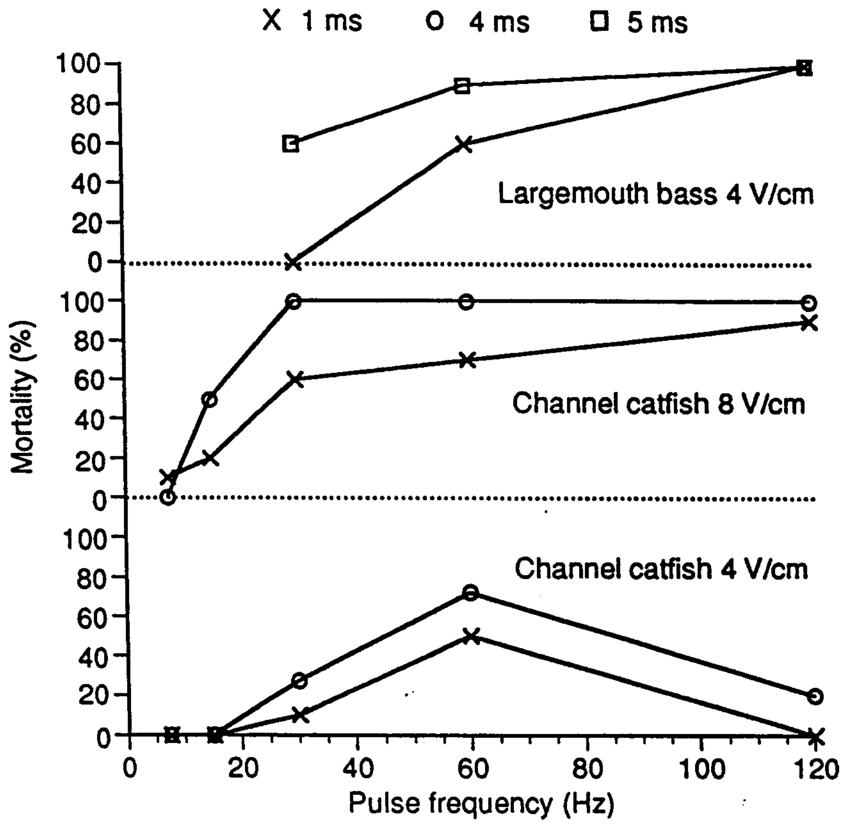


Figure V-3. Effect of pulse width on mortality of channel catfish (21-28 mm TL) and largemouth bass (21-26 mm TL) after a 20-s exposure to electric fields with different frequencies of 1- to 5-ms in water with 98-102 μS/cm ambient conductivity.

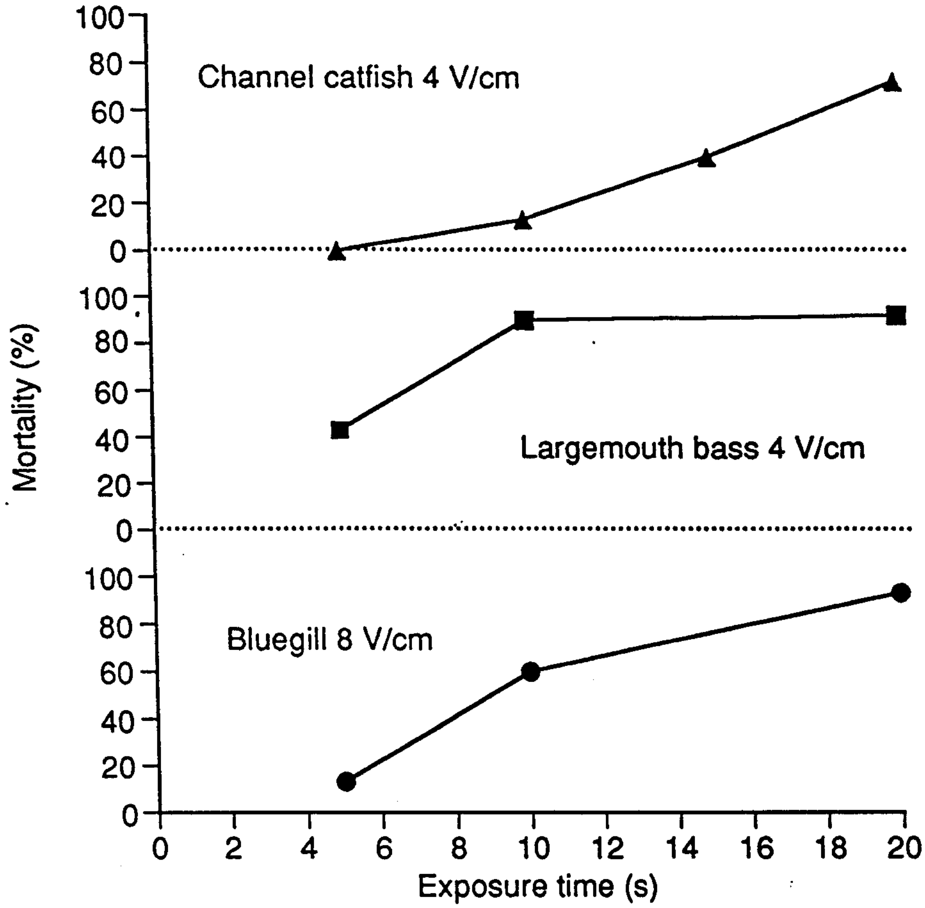


Figure V-4. Effect of exposure duration on mortality of channel catfish (24-28 mm TL), largemouth bass (22-29 mm TL), and bluegill (13-17 mm TL) 1 h after exposure to 60-Hz PDC (4 or 8 V/cm) in water with 98-120 µS/cm ambient conductivity.

VI. ELECTROSHOCKING-INDUCED MORTALITY OF FISH IN WATERS OF DIFFERENT CONDUCTIVITY

Abstract.-Water conductivity affects electric field characteristics and the ability to sample fish by electrofishing. In three experiments, each with three species of juvenile fish, we determined the immediate mortality after electroshocking with 60-Hz pulsed DC in waters of different ambient conductivity. For experiment 1, the applied power density was maintained constant (960-4900 $\mu W/cm^3$ depending on species) over a range of water conductivities (10-1020 $\mu S/cm$); in experiment 2, the voltage gradient was maintained constant (peak voltage, 2.5-8 V/cm) over the same range of conductivities; and in experiment 3, pulse width was adjusted (1, 3, or 5 ms) to electroshock in waters of different conductivity (495, 165, or 99 $\mu S/cm$) with the same peak voltage gradient and mean current density. At constant applied power, models of fish mortality predicted that the highest fish mortality would occur at water conductivities of 80 $\mu S/cm$ for bluegill *Lepomis macrochirus*, 60 $\mu S/cm$ for largemouth bass *Micropterus salmoides*, and at 140-200 $\mu S/cm$ for channel catfish *Ictalurus punctatus*. At constant voltage, fish mortality increased with current density, which is directly related to water conductivity. Fish mortality did not differ in waters with different conductivity when pulse width was adjusted to maintain a constant mean current while electroshocking fish at the same voltage level, suggesting that

the effect of increases in peak current and power density were offset by reductions in pulse width.

Introduction

Water conductivity can range from less than 20 μS/cm to over 1000 μS/cm in waters where electrofishing is conducted, and is the most important environmental variable affecting the waterborne electric field. Voltage gradient and current density are related by water conductivity, and as water conductivity varies, changes in current density and voltage gradient alter the impact of electric fields on fish (Kolz and Reynolds 1989). The interaction between electric field variables, as influenced by water conductivity, and fish response and injury, is not well understood and should be investigated to improve electrofishing procedures.

The water conductivity where electrofishing is conducted impacts the selection of electrical output for efficient electrofishing. At constant voltage, current increases directly with water conductivity, and electric power (product of voltage and current) also increases until the power output capabilities of electrofishing equipment have been exceeded (Novotony and Priegel 1974). Some types of pulsed direct current (PDC) electrofishing equipment are outfitted with mean current (amp) meters (Smith-Root, Vancouver Washington) and the duty cycle can be adjusted to maintain a constant mean output current over a range of water conductivities while electrofishing at the same peak voltage. However, adjustment of duty cycle (from 100-50%) does not prevent the peak current from increasing as water conductivity is increased; therefore, fish are exposed to higher peak power levels (peak voltage X peak current) if voltage is kept constant.

Kolz and Reynolds (1989) suggested that the ability of electric fields to affect fish is dependent on the transfer of electric power into fish tissues, and that particular fish responses (e.g., twitch or stun) result only after specific power levels have been transferred into fish. In the power transfer theory for electrofishing, Kolz (1989) demonstrated mathematically that electric power is most efficiently transferred into fish when the water conductivity and the "effective conductivity" of the fish are equal. However, the "effective conductivity" of fish cannot be directly measured, and the power transfer theory for electrofishing has not been sufficiently evaluated experimentally.

The objective of the present study was to evaluate the importance of voltage gradient, current density, applied power density, and transferred power density on mortality of fish after electroshocking in waters of different conductivity. The fish considered were largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, and channel catfish *Ictalurus punctatus*. An additional objective was to determine effects on mortality after reducing pulse width to maintain peak voltage and mean current levels constant when electroshocking in waters of higher conductivity.

Methods

Experimental animals.-Juvenile largemouth bass, bluegill, and channel catfish were obtained from the Auburn University Fisheries Research Station, and fish were fed zooplankton, brine shrimp (*Artemia*), or fish feed (Ziegler Brothers, Gardners, Pennsylvania). Fish were kept in 40-L flow-through (0.25 L/min, well water) aquaria with the following water characteristics: alkalinity, 25 mg/L as $CaCO_3$; hardness, 23 mg/L as $CaCO_3$; temperature, 18°C; pH, 7.1;

dissolved oxygen, >6.0 mg/L; and conductivity, 80 μS/cm (in this study, ambient conductivity is reported).

Electric fields.-Electric fields were generated by an electrofishing pulse box (Coffelt, TEG-10 Proto 1, Flagstaff, Arizona) modified to be powered by standard 110 V AC and to produce DC and square-pulsed DC (PDC) electric fields. The exposure chambers were plastic troughs (38.1 X 7.7 cm, 5.1 cm deep) that had flow-through water (well water) and plastic covers. Electrodes were aluminum plates that conformed to the cross-sectional area of the trough, were separated by 30 cm, and could be moved from trough to trough. Electric fields were measured with an oscilloscope each time a fish was exposed (Tectronix THS 720A, Beaverton, Oregon). The oscilloscope had two electrically isolated channels; channel 1 was used to measure voltage amplitude, pulse frequency, and pulse width, and channel 2 was connected across a 10-ohm sampling resistor to measure the current through the tank circuit. All electrical measurements of experimental treatments were within 4% of the expected value.

Experimental design.-Two h before stocking troughs the temperature in holding aquaria was adjusted to 25°C with aquarium heaters, and all experiments were conducted at 24.3-26°C. Thirty minutes before each experiment, one fish was stocked into each plastic trough prior to electroshocking. Three experiments were conducted separately for each species. Fish handling, water chemistry, exposure duration (20 s), determination of mortality, and electrical variables (except as noted) were the same in all experiments. For each experiment, control fish were randomly selected and had the electrodes placed in the trough to simulate shocking, but the electrodes were not energized.

In experiment 1, applied power density (D_a) was maintained constant over a range of water conductivities (10-1020 µS/cm). For each water conductivity, 15 fish were randomly selected to receive 60-Hz pulsed (3 ms) DC and 10 fish served as controls. Power densities were selected to kill 60-100% of fish exposed at a water conductivity of 100 µS/cm based on preliminary experiments for each species. The voltage gradient required to produce the specific power density (D_a) was calculated for all other water conductivity levels [D_a = (voltage gradient)^2(water conductivity); (Kolz 1989)]. Bluegill [14-19 mm total length (TL), age 15-20 d] were exposed to 4900 µW/cm^3; largemouth bass (34-44 mm TL, age 45-50 d) were exposed to 960 µW/cm^3; and channel catfish (34-43 mm TL, age 50-60 d), were exposed to 1560 µW/cm^3 or 3140 µW/cm^3. Distilled water was added to the well water to produce water conductivities <80 µS/cm, and artificial sea salt (Instant Ocean Synthetic Sea Salt, Mentor, Ohio) was added to the well water to adjust water conductivity between 80 and 1020 µS/cm. Fish were stocked into troughs prior to adjustment of water conductivity in the system, and were allowed to adjust to changes in conductivity for 0.5 h before exposure to electricity. After electric exposure, fish were observed for 1 h. Fish that had not recovered opercular movement after 1 h were considered dead. After determination of mortality, fish total length was determined.

For experiment 2, voltage gradient (rather than power density) was constant over a range of water conductivities (10-1020 µS/cm). Bluegill were exposed to 4 V/cm, largemouth bass were exposed to 2.5 V/cm, and channel catfish were exposed to 8 V/cm (peak voltage gradients). The fish in experiment 2 were the same size and age as in experiment 1 except channel catfish were 43-54 mm TL (age 80-90 d). Mortality was determined as described above.

In experiment 3, the peak voltage gradient was maintained constant (8 V/cm for bluegill and channel catfish, and 3 V/cm for largemouth bass), and the water conductivity was adjusted to give the same mean current (0.24 mAmp/cm^2 for channel catfish and bluegill, 0.09 mAmp/cm^2 for largemouth bass) for each pulse width tested (1, 3, and 5 ms). Fish in experiment 3 were the same size and age as in experiment 1. The water conductivities were the same for each species: for 1 ms the water conductivity was 495 µS/cm, for 3 ms the conductivity was 165 µS/cm, and for 5 ms the conductivity was 99 µS/cm.

Statistics.-Statistical analyses were conducted with Statistical Analysis Software (SAS version 8.2; SAS Institute, Cary, North Carolina). We used stepwise logistic regression (PROC LOGISTIC) to develop logistic models to predict fish mortality for each species in all three experiments. Models that were significant predictors of fish mortality ($P < 0.05$) were used to obtain the predicted probability of fish mortality (M):

$$M = e^{u} \cdot (1 + e^{u})^{-1}, \tag{1}$$

where u is a linear function of the independent variables, which differed in each experiment.

In experiment 1, two models were selected for each species at each constant power density. For model A (Table VI-1), voltage gradient, current density, and water conductivity were considered for inclusion in the model. In model B, the independent variable was the transferred power density (D_m) computed from the equation (Kolz and Reynolds 1989):

$$D_m = D_a\,[1/2 + 1/4((c_f/c_w) + (c_w/c_f))]^{-1}, \qquad (2)$$

where D_a is the applied power density in the water ($\mu W/cm^3$), c_f is the "effective conductivity" of the fish ($\mu S/cm$), and c_w is the conductivity of the water ($\mu S/cm$). The c_f value was determined by iteration of different values and selection of the value that resulted in D_m significantly ($P < 0.05$) related to fish mortality with the highest index of rank correlation (c). The value of c indicates the percent effectiveness of the model for predicting a binary response.

In experiment 2, two models were selected for each species. For model C (Table VI-2), water conductivity and current density were considered for inclusion in the model. Model D had D_m (equation 2 based on the c_f determined in experiment 1) as the independent variable. For experiment 3, fish mortality was modelled relative to the independent variables pulse width, current density, and D_m (computed as described above).

Results

During electroshocking, all species were immobilized, and survivors generally recovered opercular movements and swimming ability within 5 min. For fish that died, recovery of opercular movements did not occur at any time during the 1-h observation period following exposure. No control fish died during experiments.

At constant D_a, voltage gradient decreased with water conductivity while current density increased (Figure VI-1). The highest observed fish mortality occurred at a water conductivity of 152 μS/cm for channel catfish exposed to 1560 $\mu W/cm^3$, 152 to 230 μS/cm for channel catfish exposed to 3140 $\mu W/cm^3$, 70.5 μS/cm for bluegill, and 80 μS/cm for largemouth bass (Figure VI-2). The observed mortality of each species was lower for water conductivities above or below the conductivity where the maximum fish mortality was observed.

For fish exposed to a constant power density (D_a) over a range of water conductivities (experiment 1), two separate models were selected to predict the probability of fish mortality (Table 1). The index of rank correlation (*c*) was > 0.74 for each model and did not differ between models for each species. In model A for all species, both water conductivity and voltage gradient were significant ($P < 0.05$) except for voltage gradient with largemouth bass ($P = 0.11$). In model B the independent variable D_m was based on the c_f calculated for each species: 60 μS/cm for largemouth bass, 80 μS/cm for bluegill, 140 μS/cm for channel catfish exposed to 1560 $\mu W/cm^3$, and 200 μS/cm for channel catfish exposed to 3140 $\mu W/cm^3$. The range of c_f values that resulted in a significant ($P < 0.05$) relation between D_m and fish mortality was 20-180 μS/cm for largemouth bass, 20-140 μS/cm for bluegill, and 40-280 for channel catfish. The improvement in the index of rank correlation (*c*) after selection of the specific c_f value (comparison of significant models with the lowest and highest values of *c*) was 0.02 for largemouth bass, 0.12 for bluegill, and 0.22 for channel catfish.

At constant applied voltage gradients (experiment 2), fish did not die at water conductivity levels below 50 μS/cm, but mortality increased with water conductivity (Figure VI-3). Current density increased with water conductivity (Figure VI-4), and the lowest current density to kill largemouth bass was 148 μAmps/cm^2 (2.5 V/cm, 370 μW/cm^3), 560 μAmps/cm^2 for bluegill (4 V/cm, 2240 μW/cm^3), and 800 μAmps/cm^2 for channel catfish (8 V/cm, 6400 μW/cm^3). The probability of fish mortality was described by two separate models for each species (Table VI-2). The index of rank correlation (c) was > 0.76 for each model and did not differ between models within species. For each species, in the first model current density was significantly related to fish mortality ($P < 0.0001$), and in the second model, D_m was significantly related to fish mortality ($P < 0.001$).

When peak voltage gradient and mean current density were held constant in waters of different conductivity by adjusting pulse width, fish mortality was similar in each water conductivity (Figure VI-5). Fish mortality in this experiment was not related ($P > 0.05$) to D_a, D_m, water conductivity, or peak current density. Mean current was held constant, but peak power density and peak current density increased with water conductivity.

Discussion

Kolz and Reynolds (1989) found that the responses (twitch, attraction, and stun) of goldfish *Carassius auratus* electroshocked in waters of different conductivity were related to water conductivity and D_a, and they proposed the power transfer theory for electrofishing to explain their observations [equation (2)]. The transfer of electrical power from one resistor to another is maximized when each resistor has equal resistance (Gray 1954), and this is the principle behind the power transfer theory in electrofishing. Electrical resistance is inversely related to electrical conductivity; however, the effective conductivity of a fish cannot be directly measured, which hinders testing of the power transfer theory for electrofishing.

In our study, the power transferred into fish (D_m) calculated from equation (2) was significantly related to fish mortality when applied electric power was maintained constant, and we predicted c_f values of 60 µS/cm for largemouth bass, 80 µS/cm for bluegill, and 140-200 µS/cm for channel catfish. These values for c_f are similar to the 83-160 µS/cm c_f values reported for goldfish after exposure to PDC (Kolz and Reynolds 1989). In each species, we found a range of c_f values for largemouth bass (20-180 µS/cm), bluegill (20-140 µS/cm), and channel catfish (40-280 µS/cm) that resulted in a significant relation between D_m and fish mortality. In channel catfish, a different c_f value was selected for each constant applied power level tested and this difference could be because all fish died at water conductivities of 152 and 230 µS/cm and limited the precision of the model generated to explain fish mortality when fish were exposed at higher power density.

Other techniques have been used to measure the conductivity of fish and results differ from the c_f values in our study and those reported by Kolz and Reynolds (1989). Ionic conductivity is responsible for the conduction of electric currents through living tissue, and different components of tissue are more or less resistant to the flow of electric currents (Sternin et al. 1972). Interstitial fluids can have high conductivity (3,000-10,000 µS/cm) while cell membranes can act as electrical insulators from the interior of the cell, which can have similar electrical conductivity to the interstitial fluids (Sternin et al. 1972). The electrical conductivity of an intact fish is probably different than the conductivity of individual tissues and more relevant to electrofishing.

Whole-body electrical conductivity of fish has been determined by comparing the resistance of a tank of water to current flow induced between cross-sectional plate electrodes with and without fish in the tank (Whitney and Pierce 1957; Sternin et al. 1972). From these analyses, values of 280-3,170 μS/cm have been reported for the electrical conductivity of fish (Sternin et al. 1972). In our study, fish c_f values above 280 μS/cm did not result in a significant relation between the power transferred (D_m) and fish mortality. The difference between values for whole-body electrical conductivity of fish reported in other studies and the c_f values we found could be because of differences in the species or size of fish considered, or the c_f value and the whole-body electrical conductivity could be completely different characteristics.

We found that fish mortality at constant applied power over a range of water conductivities could also be explained by the independent variables water conductivity and voltage gradient. This model is simpler than that proposed in the power transfer theory for electrofishing, does not require determination of c_f, and had the same ability to predict fish mortality (the index of rank correlation was the same value for both models within a species). The power transfer theory of electrofishing has been criticized for not considering the effects of the strength of the current, duration of exposure, and rate of change of current intensity on fish (Sharber and Carothers 1988b). In addition, the name "power transfer" has been criticized because electric power itself can not be directly transferred into fish (Snyder 2000). The power transfer theory provides an explanation for the decrease in fish mortality at water conductivity levels below the c_f value; however, alternative hypotheses should be considered to explain fish responses.

One hypothesis to explain lower fish mortality at water conductivities below the c_f value could be lower current density. We found that current density was a significant predictor of fish mortality when voltage gradient was maintained constant over a range of water conductivities. This model predicted fish mortality as effectively as models based on power transfer (D_m). At low conductivity, fish mortality did not occur until a threshold current level was produced: 148 μAmps/cm^2 for largemouth bass, 560 μAmps/cm^2 for bluegill, and 800 μAmps/cm^2 for channel catfish. Therefore, an alternative to the power transfer theory for electrofishing could be that in low water conductivity, fish mortality is related to current density, but in high water conductivity, voltage gradient is the important variable. The channel catfish in experiment 2 (constant voltage, 8 V/cm) were more resistant to electroshocking-induced mortality than in experiment 1 (constant power) or experiment 3 (constant voltage and mean current density) probably because they were larger fish (Chapter IV).

Some operators of electrofishing equipment adjust duty cycle to maintain a constant mean current and total voltage output when electrofishing in waters of different conductivity. In experiment 3, we found that there was no difference in fish mortality relative to changes in peak current density, applied power, or transferred power when voltage and mean current were kept constant by adjusting pulse width in waters of different conductivity. Experiment 2 demonstrated that when peak current density increased, fish mortality increased, but in that experiment, pulse width was maintained constant so that mean current also increased. In experiment 3, peak D_a and peak current density increased as pulse width decreased, and mortality was predicted by mean D_a or mean current density rather than peak values, although water conductivities < 99 μS/cm were not considered. We found that shorter pulse widths resulted in lower mortality of fish electroshocked with different pulse frequencies including 60-Hz PDC (Chapter V); however, Collins et al. (1954) did not find an effect on mortality of chinook salmon *Oncorhynchus tshawytscha* after exposure to 20- or 80-ms pulse widths of 8-Hz PDC. Other studies on the effect of pulse width on fish mortality have not been conducted.

The results of experiment 3 indicate that electrofishing with constant peak voltage and adjusting duty cycle to maintain a constant mean current in waters of different conductivity, will have a consistent effect on fish mortality in the water conductivities we tested. At water conductivities lower than 99 μS/cm, the effect on mortality of increasing pulse width to maintain constant mean current is unknown. When half or full wave rectified AC (rather than square PDC) is used in PDC electrofishing, the pulse shape changes asymmetrically as duty cycle is adjusted, introducing an additional variable that could influence the fish response. The effect of changing duty cycle of half or full wave-rectified AC on fish mortality is unknown.

We found that electroshocking-induced mortality of juvenile largemouth bass, bluegill, and channel catfish in waters of different conductivity can be predicted by the power transfer theory for electrofishing (Kolz and Reynolds 1989); however, fish mortality can be predicted as effectively with simpler models that incorporate voltage gradient, water conductivity, or current density. Our results indicate that if pulse shape and electric power or voltage output are constant, the probability of fish mortality will change in waters of different conductivity; therefore, electric power or voltage output alone are not good predictors of fish mortality.

Table VI-1. Maximum-likelihood parameter estimates and associated χ^2 statistics for two logistic models (A and B) selected to predict fish mortality after exposure to electric fields with constant power density in waters of different conductivity. Independent variables were water conductivity and voltage gradient in model A, and transferred power density (D_m) in model B. The value for c_f was determined from equation 2. An index of rank correlation (*c*) indicates the percent effectiveness of the model for predicting a binary response.

Parameters	Estimate	SE	Wald χ^2	*P*	*c*
		Bluegill 4900 $\mu W/cm^3$ (model A)			
Intercept	2.5003	0.8978	7.76	0.0054	
Conductivity	-0.0065	0.00192	11.42	0.0007	
Voltage gradient	-0.1356	0.0665	4.17	0.0412	0.763
		Bluegill 4900 $\mu W/cm^3$ (model B)			
Intercept	-3.0994	0.7451	17.30	<0.0001	
D_m ($c_f = 80$)	0.00089	0.000207	18.50	<0.0001	0.763
		Largemouth bass 960 $\mu W/cm^3$ (model A)			
Intercept	2.7585	0.7239	14.52	0.0001	
Conductivity	-0.00575	0.00140	16.86	<0.0001	
Voltage gradient	-0.2129	0.1346	2.50	0.1138	0.742
		Largemouth bass 960 $\mu W/cm^3$ (model B)			
Intercept	-2.3498	0.6263	14.08	0.0002	
D_m ($c_f = 60$)	0.00439	0.000852	26.61	<0.0001	0.742

(continued)

Table VI-1. Continued

Parameters	Estimate	SE	Wald χ^2	*P*	*c*
	Channel catfish 1560 $\mu W/cm^3$ (model A)				
Intercept	4.2342	1.0681	15.71	<0.0001	
Conductivity	-0.00923	0.0023	16.08	<0.0001	
Voltage gradient	-0.6615	0.1638	16.31	<0.0001	0.783
	Channel catfish 1560 $\mu W/cm^3$ (model B)				
Intercept	-4.7710	0.9554	24.94	<0.0001	
D_m ($c_f = 140$)	0.0036	0.000751	22.94	<0.0001	0.783
	Channel catfish 3140 $\mu W/cm^3$ (model A)				
Intercept	5.4889	1.0800	25.83	<0.0001	
Conductivity	-0.00482	0.00111	18.72	<0.0001	
Voltage gradient	-0.5108	0.1150	19.74	<0.0001	0.807
	Channel catfish 3140 $\mu W/cm^3$ (model B)				
Intercept	-0.31062	0.8633	12.95	0.0003	
D_m ($c_f = 200$)	0.00191	0.000422	20.45	<0.0001	0.807

Table VI-2. Maximum-likelihood parameter estimates and associated χ^2 statistics for two logistic models (C and D) selected to predict fish mortality after exposure to electric fields with constant voltage gradient in waters of different conductivity. Independent variables were current density in model C, and transferred power density (D_m) in model D. The value for c_f was determined from equation 2. An index of rank correlation (*c*) indicates the percent effectiveness of the model for predicting a binary response.

Parameters	Estimate	SE	Wald χ^2	*P*	*c*
			Bluegill 4 V/cm (model C)		
Intercept	-2.6249	0.4456	34.70	<0.0001	
Current	0.0714	0.0144	24.46	<0.0001	0.910
			Bluegill 4 V/cm (model D)		
Intercept	-4.7767	0.9449	25.55	<0.0001	
D_m (c_f = 80)	0.00149	0.000290	26.57	<0.0001	0.910
			Largemouth bass 2.5 V/cm (model C)		
Intercept	-2.4333	0.5727	18.05	<0.0001	
Current	0.3552	0.0886	16.06	<0.0001	0.927
			Largemouth bass 2.5 V/cm (model D)		
Intercept	-4.3132	0.9622	20.09	<0.0001	
D_m (c_f = 60)	0.00671	0.00141	22.72	<0.0001	0.927
			Channel catfish 8 V/cm (model C)		
Intercept	-1.9990	0.4720	17.94	<0.0001	
Current	0.0168	0.00514	10.63	<0.0001	0.762

(continued)

Table VI-2. Continued.

Parameters	Estimate	SE	Wald χ^2	*P*	*c*
		Channel catfish 8 V/cm (model D)			
Intercept	-2.6408	0.6531	16.35	<0.0001	
D_m ($c_f = 140$)	0.000117	0.000035	11.24	<0.0001	0.762

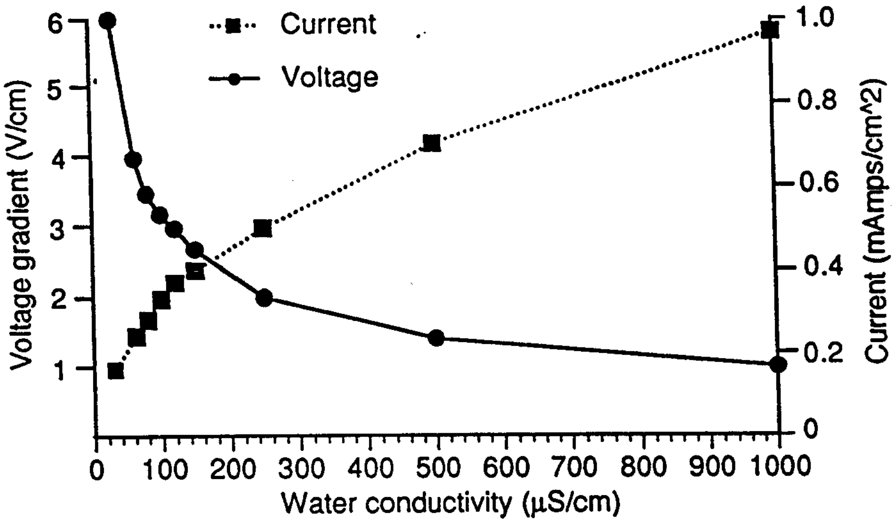
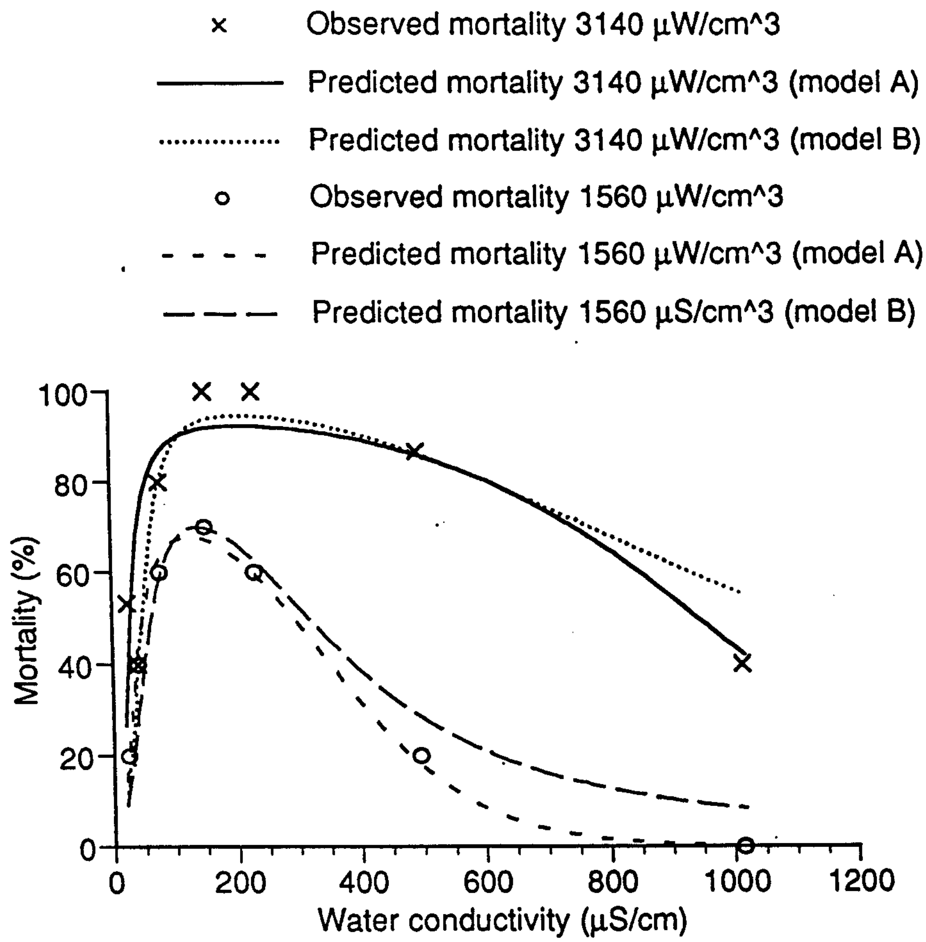


Figure VI-1. Relation of voltage gradient, current density, and water conductivity when power density is maintained constant (1000 $\mu W/cm^3$).

Figure VI-2. Electroshocking-induced mortality of fish after exposure (20 s) to 60-Hz PDC at constant power density over a range of water conductivities. (a) Channel catfish exposed to 1560 or 3140 $\mu W/cm^3$, (b) bluegill exposed to 4900 $\mu W/cm^3$, and (c) largemouth bass exposed to 960 $\mu W/cm^3$. Models of fish mortality are presented in Table VI-1.



× Observed mortality 3140 μW/cm^3
Predicted mortality 3140 μW/cm^3 (model A)
Predicted mortality 3140 μW/cm^3 (model B)
o Observed mortality 1560 μW/cm^3
Predicted mortality 1560 μW/cm^3 (model A)
Predicted mortality 1560 μS/cm^3 (model B)
Mortality (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
Water conductivity (μS/cm)

Figure VI-2a.

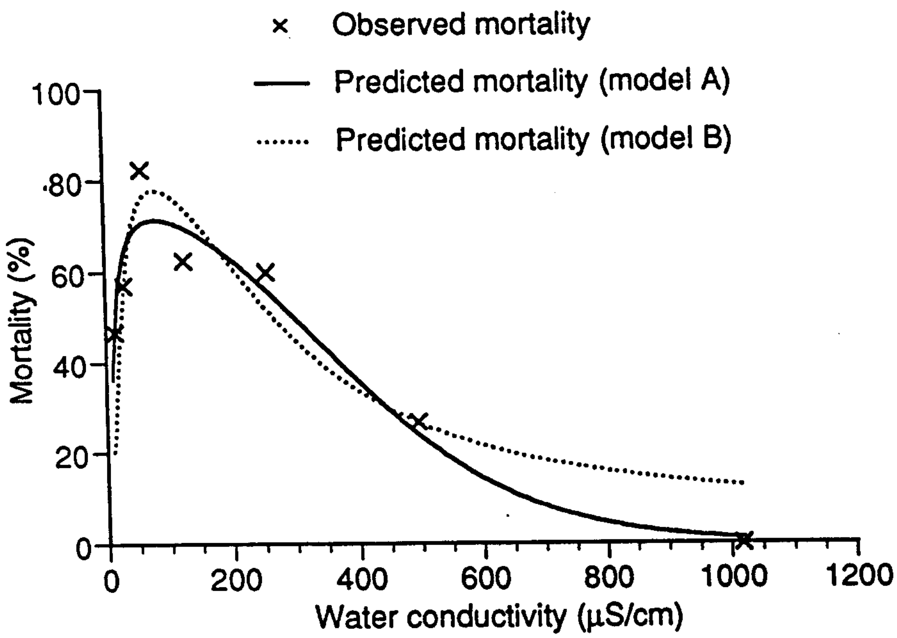
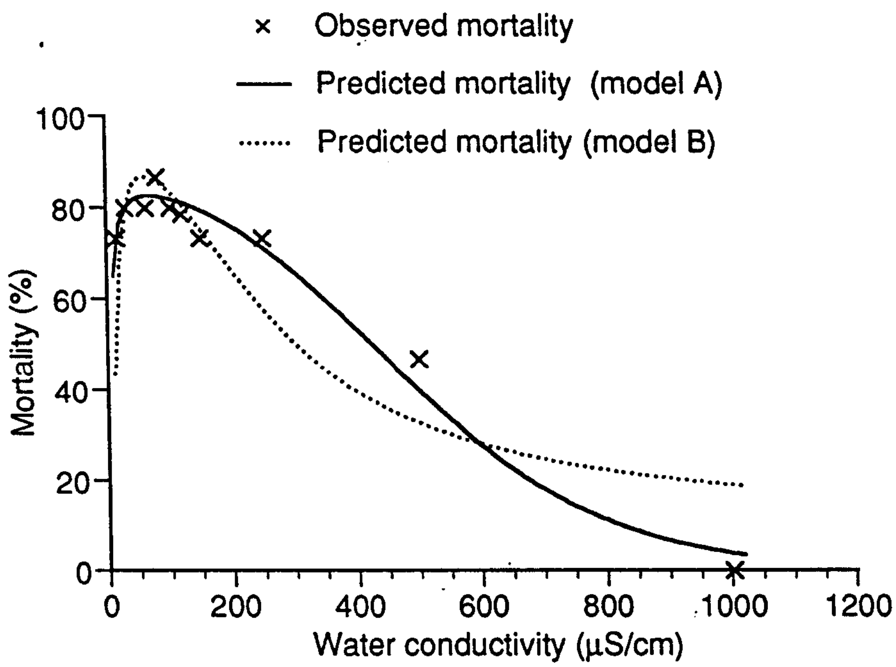

× Observed mortality
Predicted mortality (model A)
Predicted mortality (model B)
Mortality (%)
100
80
60
40
20
0
0
200
400
600
800
1000
1200
Water conductivity (μS/cm)

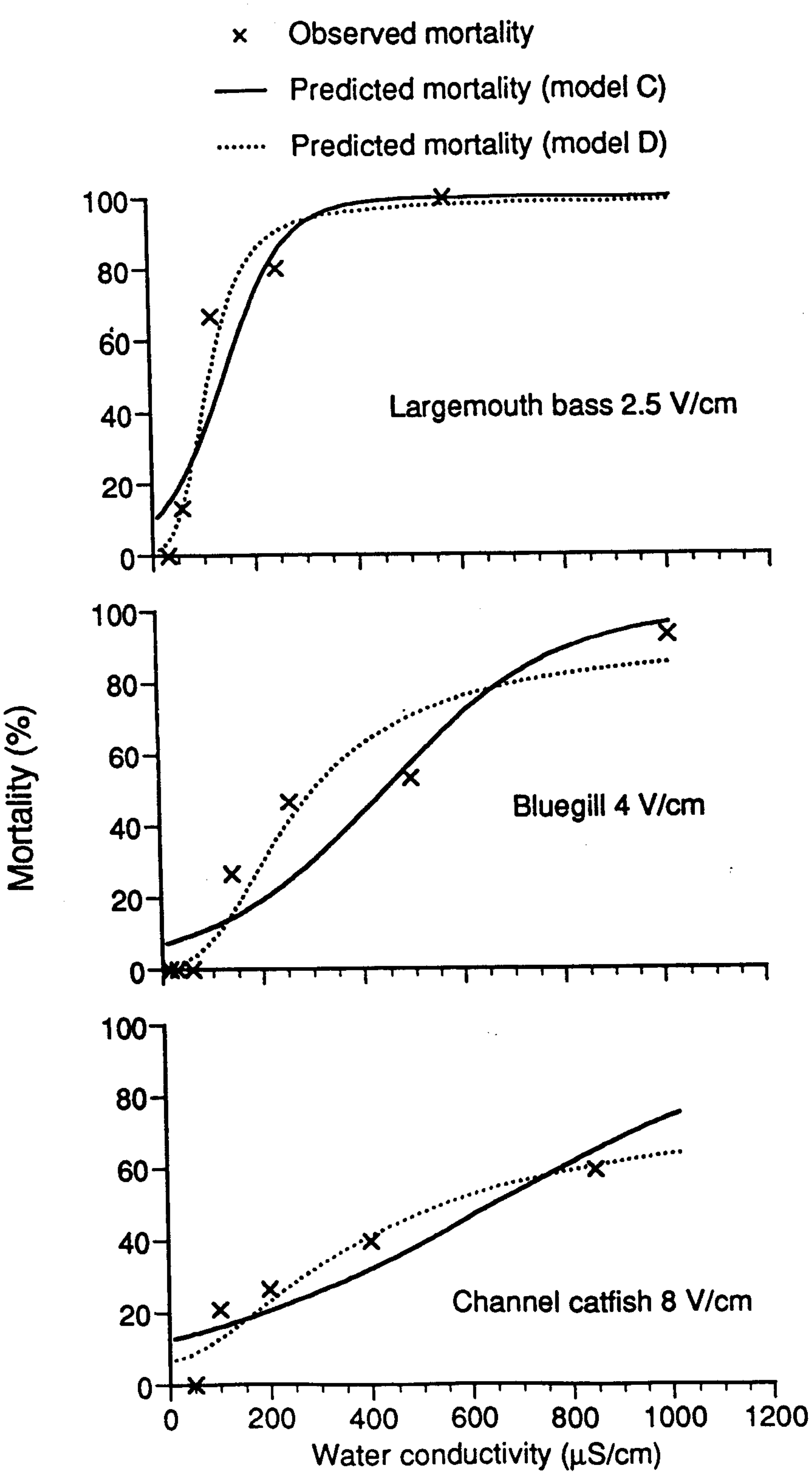
Figure VI-2b.



× Observed mortality
Predicted mortality (model A)
Predicted mortality (model B)
100
80
60
40
20
0
Mortality (%)
0
200
400
600
800
1000
1200
Water conductivity (µS/cm)

Figure VI-2c.

Figure VI-3. Fish mortality after exposure (20 s) to constant voltage gradients of 60-Hz PDC over a range of water conductivities. (a) Largemouth bass exposed to 2.5 V/cm, (b) bluegill exposed to 4 V/cm, and (c) channel catfish exposed to 8 V/cm. Models of fish mortality are presented in Table VI-2.



× Observed mortality
Predicted mortality (model C)
Predicted mortality (model D)
Largemouth bass 2.5 V/cm
Bluegill 4 V/cm
Channel catfish 8 V/cm
Mortality (%)
0
20
40
60
80
100
Water conductivity (µS/cm)
0
200
400
600
800
1000
1200

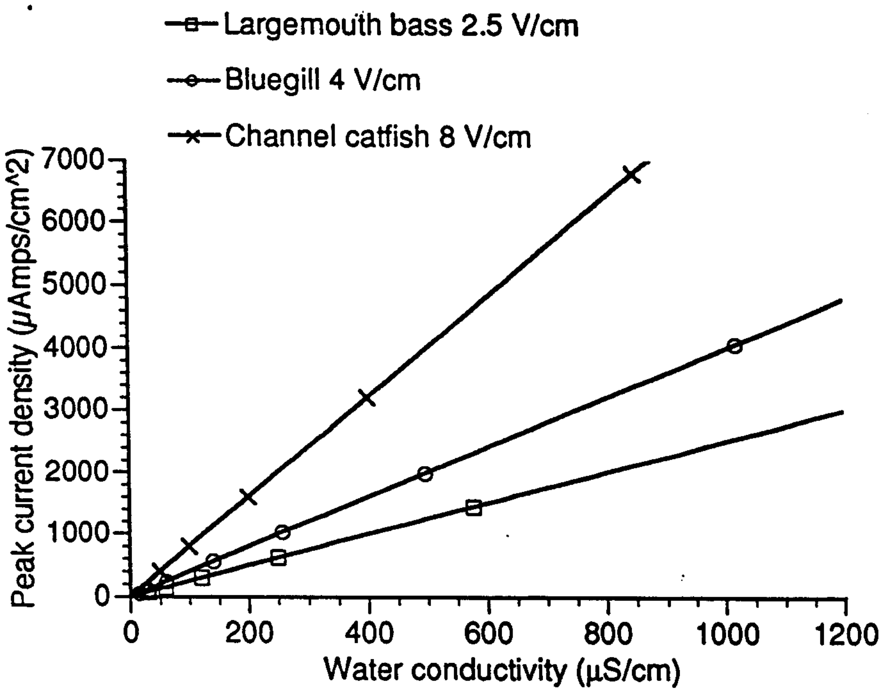


Figure VI-4. Relation of current density to water conductivity when fish were exposed to constant voltage gradients.

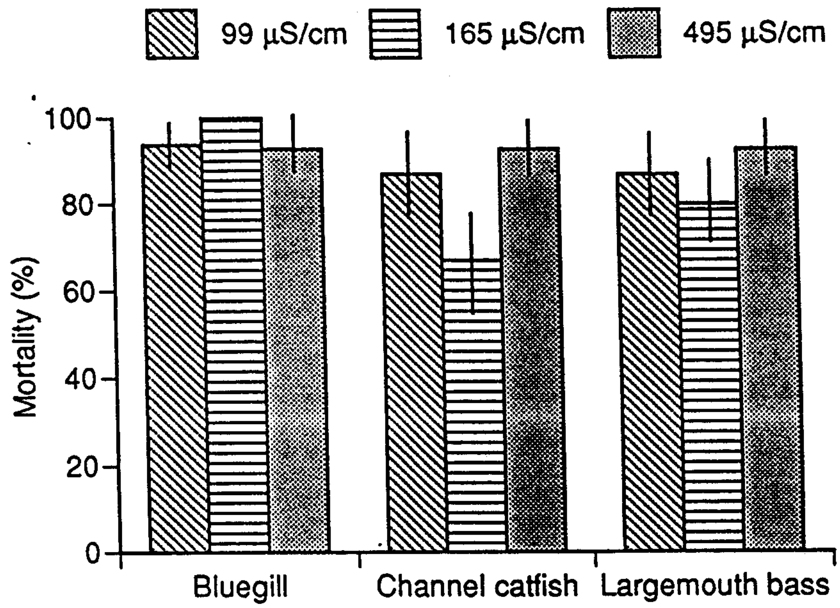


Figure VI-5. Mortality (vertical line = SE) of bluegill, channel catfish, and largemouth bass after exposure to constant peak voltage gradient and mean current density in water with different conductivity. Constant mean current was obtained in different conductivity by changing pulse widths of 60-Hz PDC. Pulse widths used were 5 ms for 99 µS/cm, 3 ms for 165 µS/cm, and 1 ms for 495 µS/cm. For largemouth bass, voltage gradient was 3 V/cm and mean current density was 0.09 mAmp/cm^2; and for bluegill and channel catfish voltage gradient was 8 V/cm and mean current density was 0.24 mAmps/cm^2.

VII. ELECTROSHOCKING-INDUCED INJURIES IN NEWLY TRANSFORMED JUVENILE FISH

Abstract.-Fish that had recently become juveniles were electroshocked and then examined for gross and microscopic lesions. Species examined were largemouth bass *Micropterus salmoides*, bluegill *Lepomis macrochirus*, striped bass *Morone saxatilis*, channel catfish *Ictalurus punctatus*, Nile tilapia *Oreochromis niloticus*, and rainbow trout *Oncorhynchus mykiss*. Fish were exposed for 5 s (bluegill only) or 20 s to homogeneous electric fields of 30-, 60, or 120-Hz pulsed DC at voltage gradients of 2-16 V/cm in water with 70-100 μS/cm ambient conductivity. Gross injuries occurred in all species, but only 2.8% of the survivors (N = 787) and 0.7% of fish that died immediately after electroshocking (N = 598) had gross injuries. Hemorrhage in the posterior trunk or anterior tail was the most common gross lesion, occurring in 17 fish; and histological examination revealed that 8 of 9 fish had a fractured vertebra or notochord hernia at the location of hemorrhage. For three species, fish that survived electroshocking without gross injuries were examined histologically. An injured vertebra or herniated notochord occurred in 17% (N = 12) of bluegill exposed for 5 s (8 V/cm) and in 40% (N = 15) of channel catfish (4 V/cm), but no vertebral injuries occurred in 16 bluegill exposed for 20 s (4 V/cm) or in 22 largemouth bass (2 V/cm). Necrotic skeletal muscle fibers occurred in 60% of channel catfish, 63-67% of bluegill (depending on voltage gradient and duration), and 18% of largemouth bass that survived

electroshocking without gross injuries. Necrotic muscle was also found in one control largemouth bass (8%) and one control bluegill (20%). Results indicate that after electroshocking, lesions are seldom visible grossly, gross and microscopic injuries are as likely to occur in survivors as in fish that died, and the frequency of microscopic lesions can be high in newly transformed fish.

Introduction

The potential for electric fields produced by electrofishing equipment to injure fish is well documented (Hauck 1949; Reynolds 1996), and injuries can occur in many different fish species (Snyder 1992). However, no information exists on the frequency, severity, and types of injuries occurring in recently transformed juvenile fish, which was the developmental period most susceptible to electroshocking-induced mortality in the four species studied (Chapter IV). Sublethal, electroshocking-induced injuries in older fish have been linked to decreased swimming ability (Horak and Klein 1967; Mitton and McDonald 1994), vertebral lesions (Dalbey et al. 1996; Kocovsky et al. 1997; Thompson et al. 1997a), and reduced growth (Gatz et al. 1986; Thompson et al. 1997b).

Evaluations of the negative effects of electroshocking on fish have most commonly involved external examination and necropsy (Pratt 1955; Spencer 1967a; Ruppert and Muth 1997), radiography (Hudy 1985; Thompson et al. 1997a), and analysis of blood (Schreck et al. 1976; Burns and Lantz 1978; Barton and Dwyer 1997). Except for one study that considered only one shocked fish and did not find any lesions (Taylor et al. 1957), histopathology of fish injured by electroshocking has not been conducted. Histological assessment of electric injuries in terrestrial vertebrates has been useful to describe injuries in affected tissues (Lee et al. 2000), and could improve our understanding of electroshocking-induced injuries in fish. Small fish are particularly suitable for histopathology because the whole fish can be serially sectioned for examination of all organs.

Investigations of electroshocking-induced injuries in fish have focused mostly on hemorrhage and spinal damage (Reynolds 1996). These injuries have been related to spasmodic contractions of skeletal muscles that generate compression on the vertebrae, resulting in misalignment and fracture of vertebrae and damage to adjacent tissues (Lamarque 1990). High rates of spinal injury have been reported in some electrofishing situations (Sharber and Carothers 1988a), and restrictions have been placed on the use of electrofishing in some areas (Schill and Belland 1995). Although vertebral injuries have been documented in older juvenile and adult fish, the susceptibility of recently transformed juveniles is unknown.

The objective of the present study was to determine the frequency and severity of electroshocking-induced injuries in recently transformed juvenile fish. Fish examined were largemouth bass *Micropterus salmoides,* bluegill *Lepomis macrochirus,* channel catfish *Ictalurus punctatus,* Nile tilapia *Oreochromis niloticus,* striped bass *Morone saxatilis,* and rainbow trout *Oncorhynchus mykiss.* The frequency of grossly visible lesions was determined, and histopathology was used to evaluate lesion severity and to determine the frequency and severity of lesions that were not visible grossly.

Methods

Experimental fish.-Young juvenile fish used in experiments included: largemouth bass, bluegill, channel catfish, and Nile tilapia obtained from the Auburn University Fisheries Research Station, striped bass from the McDuffie Hatchery (Georgia Department of Natural Resources), and rainbow trout from Buffalo Springs Hatchery (Tennessee Wildlife Resources Agency). Fish were kept in 40-L aquaria with flow-through well water (0.25 L/min) with the following characteristics: alkalinity, 25 mg/L as $CaCO_3$; hardness, 23 mg/L as $CaCO_3$; pH, 7.1; dissolved oxygen, > 6.0 mg/L; conductivity, 80 µS/cm; and temperature, 18°C. Fish were fed zooplankton, brine shrimp (*Artemia*), and powdered fish feed.

Electric fields.-Exposure chambers were plastic troughs (38.1 X 7.7 cm, and 5.1 cm deep) for all species except rainbow trout, which were exposed in 40-L glass aquaria. Electricity was delivered to the water with aluminum plate electrodes, which conformed to the cross-sectional area of the troughs or glass aquaria. The electrodes were separated by 30 cm in troughs and 45 cm in aquaria and could be moved from chamber to chamber. Electric fields were measured with an oscilloscope each time a fish was exposed (Tectronix THS 720A, Beaverton, Oregon). The oscilloscope was used to measure voltage amplitude, pulse frequency, and pulse width; and all electrical measurements of experimental treatments were within 4% of the expected value. The voltage amplitude was used for determining the voltage gradient.

Except for rainbow trout, which were electroshocked in water at 18.5°C and with 70 µS/cm ambient conductivity, all electroshocking experiments were conducted at 24-26°C and 98-102 µS/cm ambient conductivity; water conductivity was adjusted by adding artificial sea salts (Instant Ocean

Synthetic Sea Salt, Mentor, Ohio). Prior to experiments, homogeneity of the electric field in the plastic troughs and the glass aquaria was tested with the oscilloscope connected to a sampling electrode (Henry et al., in press), and voltage gradients were found to be uniform throughout the exposure chambers.

Experimental design.-The exposure chambers had flow-through water (well water) exchange and plastic covers. Prior to stocking troughs, the water temperature in the holding aquaria was adjusted to 25°C with aquarium heaters for 2-3 h, and fish were stocked (1 fish/trough) 20 min before electroshocking. Rainbow trout were stocked into 40-L glass aquaria 7 d prior to electroshocking, and water temperature was not adjusted. During stocking, each fish was examined, and fish with apparent deformity, injury, or abnormal behavior were replaced. Fish in exposure chambers were randomly selected to receive an electric shock or to serve as controls. Control fish were treated the same as fish that received an electric shock except the electrodes were not energized. Electroshocked fish were exposed to 30- to 120-Hz square pulsed (3 ms) DC at 2-16 V/cm for 20 s, and immediate (1 h) mortality was evaluated. For bluegill, fish were also exposed for 5 s to 60-Hz PDC at 8 V/cm.

After determination of mortality, fish were examined for the presence of grossly visible injuries. Nine fish that survived electroshocking and had gross injuries were kept in exposure troughs or aquaria and observed for 24 h. At the end of the observation period (1 h or 24 h), the total length was determined and each fish was preserved in Bouin's fixative for histological examination. After tissues were in fixative for 48 h, they were stored in 50% isopropyl alcohol. In most fish the head and caudal fin were removed prior to tissue processing and embedding in paraffin. Sagittal or frontal serial sections (5 μm thick) were cut, mounted on slides, stained with hematoxylin and eosin (Humason 1979), and examined by light microscopy. Sections of whole fish were examined for 37 fish, while examinations of 80 fish sectioned without the head included only the trunk and tail.

The frequency of injuries that were not visible grossly was determined only in largemouth bass, bluegill, and channel catfish; and all fish examined histologically were preserved 1 h after exposure. Largemouth bass were 15.1-29.9 mm TL and age 20-40 d after hatching, bluegill were 14.3-22.2 mm TL and 15-25 d, and channel catfish were 15.8-39.4 mm TL and age 18-51 d. Fish that survived electroshocking and fish that died were examined. For fish that survived, largemouth bass were exposed to 2 V/cm 60-Hz PDC and bluegill and channel catfish were exposed to 4 V/cm 60-Hz PDC. Dead fish were exposed to 60-Hz PDC at 4 or 8 V/cm.

Results

Survival.-All fish were immobilized during electroshocking. Surviving fish had recovered opercular movement and swimming ability within 5 min. If opercular movement did not resume within 1 h, fish were considered dead. Survival after electroshocking ranged from 32 to 86%, depending on size and species (Table VII-1), and no control fish of any species died during experiments.

Smaller fish were translucent so that some types of internal injuries (e.g., hemorrhage around vertebrae) were visible; however, fish >40 mm TL were often sufficiently opaque to prevent detection of internal injuries. Fish size was not important for evaluations of uncoordinated swimming, paralysis, or scoliosis.

Grossly visible injuries.-No control fish had grossly visible injuries. Of the 787 fish that survived electroshocking, 22 fish (2.8%) had grossly visible injuries; and of the 598 fish that died, only four channel catfish (0.7%) had grossly visible injuries (Table VII-2). Paralysis or uncoordinated swimming were observed in 8 survivors, but these indications of injury were not relevant during examination of fish that died after electroshocking. Gross injuries occurred in each of the six species, although larger bluegill (24-44 mm TL) and largemouth bass (30-58 mm TL) did not have gross injuries. For species exposed to different electric current types, the frequency of injury was too low to determine which electric currents were more likely to cause gross injuries.

The 26 fish (dead and alive) with gross injuries included 17 fish with hemorrhage in the posterior trunk or anterior tail, five fish with uncoordinated swimming, three fish with scoliosis (each of which also had hemorrhage), and three fish with paralysis of posterior trunk and caudal myomeres (Table VII-2). In one rainbow trout (36 mm TL), dark skin pigmentation occurred posterior to the mid-trunk region, but this fish had no other gross indications of injury. One bluegill had hemorrhage in the anterior cranium in addition to hemorrhage around anterior caudal vertebrae. Seven fish with gross injuries survived in exposure chambers for a 24-h observation period and these injuries included: rainbow trout (65-122 mm TL) with paralysis of posterior trunk and caudal myomeres (3 fish) or uncoordinated swimming (1 fish), and bluegill with hemorrhage around vertebrae near the junction of trunk and tail (1 fish) or uncoordinated swimming (1 fish).

Histopathology.-Serial sections were examined for 15 fish with gross indications of injury (Table VII-3) and for 102 fish (including 21 controls) that did not have gross signs of injury (Table VII-4). Except for rare necrotic muscle fibers (see below), lesions were not found in the 21 control fish examined (Figure VII-1).

Vertebral fractures were found in three fish with gross indications of injury and six fish without gross indications of injury after electroshocking. Only one vertebra was fractured in each fish, and the injured vertebra was located in the posterior trunk or anterior tail (Figure VII-2). In all fish with a fractured vertebra, compression and subluxation of vertebrae were observed anterior and posterior to the fractured vertebra (Figure VII-3 and 4). For fish with and without gross injuries, compression and subluxation of vertebrae were also observed in 14 fish that did not have vertebral fractures (Table VII-3 and 4). In all other electroshocked fish, the appearance of vertebrae did not differ from control fish, which were slightly compressed but did not have subluxation of vertebrae or compaction of the notochord. Two of the three fish with scoliosis (Table VII-2) were examined histologically, and the striped bass had a fractured vertebra while the channel catfish had compression and subluxation of vertebrae but no vertebral fracture. The frequency of vertebral fractures in fish that survived electroshocking without gross indications of injury was 13% in channel catfish exposed to 4 V/cm and 17% in bluegill exposed to 8 V/cm (5 s duration); however, fractures did not occur in sampled bluegill exposed to 4 V/cm or largemouth bass exposed to 2 V/cm.

After electroshocking, herniation of the notochord was found in three rainbow trout, 10 channel catfish, and two bluegill (Table VII-3 and 4). All fish with notochord hernias had vertebral compression and subluxation of vertebrae. Notochord hernias occurred between vertebrae in all fish except for one bluegill and 5 channel catfish that had herniation through a vertebral fracture. Two of the rainbow trout with paralysis of myomeres (Table VII-2) were examined histologically; in both fish the notochord protruded dorsally between anterior trunk vertebrae, entered the spinal cord, and the spinal cord was necrotic (Figure VII-5). The third rainbow trout had dark pigmentation posterior to the mid-trunk, and herniated notochord protruded ventrally into the trunk kidney (Figure VII-6). For the channel catfish and bluegill, herniation occurred in the posterior trunk or anterior tail and the notochord was extruded dorsally, ventrally, or laterally (Figure VII-7). Notochord herniation occurred in 4 channel catfish (27%) and 1 bluegill (8%; 8 V/cm) that survived electroshocking without gross indications of injury (Table VII-4). The sampled bluegill exposed to 4 V/cm and largemouth bass exposed to 2 V/cm did not have herniation of the notochord (Table VII-4).

Nine of the 17 fish with grossly visible hemorrhage (Table VII-2) were examined histologically. Grossly visible hemorrhage was associated with fractured vertebra in one striped bass and two channel catfish, and with herniated notochord in four channel catfish and one bluegill. One largemouth bass had gross hemorrhage in the anterior tail after electroshocking and necrosis of 5-10 skeletal muscle fibers on histological examination, but no injured vertebrae. Gross hemorrhage in the cranium occurred in one bluegill (Figure VII-8) that survived electroshock (hemorrhage was also visible grossly in the anterior tail), but was not found in the crania of any other fish grossly, or in 37 heads examined histologically.

In fish that survived electroshocking without gross indications of injury, hemorrhage was observed during histological examination of three bluegill (8 V/cm), two channel catfish, and two largemouth bass (Table VII-4). The channel catfish and two of the bluegill also had vertebral injury and/or notochord herniation. The remaining bluegill, and two largemouth bass, had hemorrhage located in skeletal muscle myomeres of the trunk or tail with several necrotic muscle fibers but no other lesions.

The most common injury detected histologically in electroshocked fish was necrosis of skeletal muscle fibers (Figure VII-9). Necrotic muscle fibers had a vacuolated appearance with coagulation and banding of cytoplasm and pyknotic or karyorrhectic nuclei. Histological examination of dead and surviving electroshocked fish revealed muscle necrosis in 13 of the 15 fish with gross injuries (Table VII-3), and 41 of the 81 fish without grossly visible injuries (Table VII-4). Generally, fish had fewer than 10 necrotic muscle fibers, which commonly occurred separately in the trunk and caudal muscles. An exception was multifocal necrosis of muscle fibers in consecutive myomeres of two Nile tilapia that had uncoordinated swimming after electroshocking. These two Nile tilapia did not have other microscopic lesions. Two bluegills, one with grossly visible hemorrhage and the other with uncoordinated swimming, were preserved 24 h after exposure and had necrotic muscle fibers infiltrated by inflammatory cells (Figure VII-10). In fish that survived electroshocking without gross injuries, necrotic muscle was found in 18% of largemouth bass, 63-67% (depending on voltage gradient and duration) of bluegill, and 60% of channel catfish.

Necrosis of skeletal muscle fibers was found in two of 21 control fish that were exposed to the same handling but not to electricity (Table VII-4). In one largemouth bass control, necrosis of a skeletal muscle fiber was found in the adductor mandibulae muscle (located in the head; Figure VII-11). The appearance of this necrotic muscle was similar to necrotic muscle in electroshocked fish preserved 1 h after exposure. In one bluegill control, multifocal necrosis of muscle fibers occurred; however, extensive shrinkage of skeletal muscle fibers was also visible (Figure VII-12), and necrotic cells did not have the banding pattern seen in necrotic fibers in electroshocked fish.

For all 117 fish examined histologically, lesions were not found in any other organs than mentioned above. Based on histopathology, some electroshocked fish that died without grossly visible injuries (Table VII-4) had fractured vertebrae (12.5%), notochord herniation (12.5%), and necrosis of skeletal muscle fibers (63%). Of the electroshocked fish that died without gross injuries, 38% did not have lesions detected by histopathology.

Discussion

Electroshocking-induced injuries in newly transformed juvenile fish have not been described previously. Our results for grossly visible injuries in fish after electroshocking agree with studies of older juvenile and adult fish, in which there was no relation between the presence of grossly visible injury and death after electroshocking (Spencer 1967a; Hudy 1985; Habera et al. 1996). For microscopic examinations of electroshocked fish, we found similar lesions in fish that survived electroshocking and in fish that died, and 38% of the fish that died did not have microscopic lesions. This suggests that the microscopic lesions we found were also not related to fish mortality.

Most previous studies of electroshocking-induced injury considered lesions in vertebrae and associated hemorrhage in fish collected during field electrofishing (Hauck 1949; Sharber and Carothers 1988a; Hollender and Carline 1994). The voltage gradient and exposure duration to which fish are exposed are generally unknown during field electrofishing conditions, which hinders comparison of injury rates among studies. One study reported that no vertebral injury occurred in rainbow trout after electroshocking (McCrimmon and Bidgood 1965), while other studies with the same species have found more than 50% of fish had injured vertebrae (Sharber and Carothers 1988a; Dalbey et al. 1996). While the frequency of vertebral injury can increase with fish size (Hollender and Carline 1994), the smallest fish that have been reported with vertebral injuries after electroshocking were 45-63 mm TL greenside darters *Etheostoma blenniodes* (Cooke et al. 1998).

We found vertebral lesions (including notochord hernias) in 40% of the channel catfish that survived electroshocking without gross injury, but did not find injured vertebrae in bluegill exposed to the same electrical treatment. This suggests that differences in susceptibility to electroshocking-induced injury exist among species. The susceptibility of bluegill, largemouth bass, and channel catfish to electroshocking-induced injury has been investigated previously (Spencer 1967a); 1.5-12.2% (depending on current type and voltage) of bluegill, 1.4% of largemouth bass, and 6 of 6 channel catfish had vertebral injuries, usually in the anterior tail. However, differences in methods limit comparison between the results of Spencer (1967a) and the present study.

We found that newly transformed juvenile fish were seldom (<3%) grossly injured after electroshocking, but some fish had high rates of injury detected by histological examination. The higher sensitivity of histopathology allowed detection of notochord herniation and muscle necrosis. The only report of notochord herniation in fish prior to our study was an anecdotal account of a grossly visible lesion in electroshocked paddlefish *Polyodon spathula* (Snyder 1992), while necrotic muscle fibers have not been reported previously in the context of electrofishing. Although notochord herniation can have negative effects on fish (including paralysis), we found herniation could occur with minimal damage to vertebrae and no hemorrhage. Previous studies used radiography to detect injury to vertebrae and gross dissection to detect hemorrhage (Hollender and Carline 1994; Habera et al. 1996); however, these techniques could overlook notochord hernias. The potential for fish to recover from notochord herniation is unknown.

Skeletal muscle necrosis in electroshocked fish occurred in association with injury to vertebrae, or was the only lesion found in some fish. Some of the necrotic muscle fibers could have resulted from mechanical damage to cells from fractured vertebrae or notochord hernias; however, necrotic muscle not associated with vertebral injuries or in fish without vertebral injury requires another explanation. One hypothesis is that excessive voltage gradients across the cell membrane induced cell lysis (Ho and Mittal 1996). In our study, bluegill that were preserved 24 h after electroshocking had necrotic muscle infiltrated by inflammatory cells, suggesting removal of cellular debris and resolution of the injury. Negative effects of necrotic muscle on fish survival likely depend on the extent of the injury, and if sufficient fibers are impacted, swimming ability could be compromised (e.g., the two Nile tilapia with uncoordinated swimming in the present study). However, all fish but the two Nile tilapia had few necrotic muscle fibers, and these were generally scattered.

While necrotic muscle fibers occurred more frequently in electroshocked fish, two fish that were not electroshocked had muscle necrosis, suggesting that mechanisms other than electroshocking could be involved. The necrotic muscle in the control largemouth bass appeared similar to the muscle necrosis in electroshocked fish, but the necrotic muscle observed in the control bluegill had less fragmented cytoplasm. Necrosis of skeletal muscle fibers in fish has been associated with nutritional, bacteriological, virological, and parasitological diseases (Ferguson 1989); and potentially, fish handling could injure muscle fibers leading to necrosis. Necrotic skeletal muscle fibers in the hind limb of rats, exposed to electricity by direct contact with electrodes (Lee et al. 2000), had the same histological appearance we observed in fish exposed to in-water electric fields.

While exposure duration has been related to fish mortality (Collins et al. 1954; Chapter V) our results and those of Spencer (1967a) indicate that even short exposure durations can induce sublethal injuries. We found that 17% of bluegill that survived without gross injury after a 5-s exposure to 8 V/cm (60-Hz PDC) had vertebral injuries and 67% had necrotic muscle fibers. Voltage gradients of 2-16 V/cm can occur between electrodes of electrofishing boats (Henry et al., in press), and results of the present study indicate that newly transformed juvenile fish can be injured by these electric fields.

Our results indicate that sublethal injuries induced by electroshocking can occur in newly transformed juvenile fish; however, validation of results in actual electrofishing conditions is necessary (e.g., Chapter IV). Long-term effects of electroshocking-induced injuries in newly transformed juvenile fish are unknown; however, in older juveniles and adults, reductions in growth (Gatz et al. 1986) and permanent deformities can occur after electrofishing (Kocovsky et al. 1997). The potential for electroshocking to injure fish has resulted in modifications of fish sampling procedures (Nielsen

1998) and limitations on the use of electrofishing in some areas (Schill and Beland 1995). Because newly transformed juvenile fish can be injured during electroshocking, modification of electrofishing procedures should be considered in areas where these fish are present.

Table VII-1. Survival of fish after electroshocking. Except for rainbow trout, which were electroshocked in water of 18.5°C and 70 μS/cm ambient conductivity, all species were electroshocked in 24-26°C water with conductivity of 98-102 μS/cm.

Species	Total length (mm)	Voltage gradient (V/cm)	Frequency (Hz)	*N*	Survival (%)
Bluegill	13-23	4-8	60	237	54
	24-44	4-8	60	74	66
Largemouth bass	15-29	2-8	60	222	32
	30-58	2-8	60	102	58
Channel catfish	16-28	4-8	60	122	47
	29-53	4-8	60	111	48
Striped bass	23-30	2-8	120	68	66
Nile tilapia	10-37	16	60	147	86
Rainbow trout	18-38	4-8	60	150	45
	65-122	4	30	152	86

Table VII-2. The number of dead and alive fish with grossly visible indications of injury after electroshocking. Information about fish and characteristics of the electric fields are given in Table VII-1.

Species	*N*	Hemorrhage	Scoliosis	Paralysis	Uncoordinated swimming
Bluegill	3	2			1
Largemouth bass	1	1			
Channel catfish[a]	7	7	1		
Striped bass	1	1	1		
Nile tilapia	4	1[b]	1		3
Rainbow trout[c]	10	5		3	1

[a]Four channel catfish died after electroshocking, all had hemorrhage.

[b]This fish also had scoliosis.

[c]One rainbow trout had loss of control of melanophores and no other indications of injury.

Table VII-3. Histopathology of fish that had grossly visible indications of injury after electroshocking. Information about fish and characteristics of the electric exposure are given in Table VII-1. The grossly visible indications of injury were uncoordinated swimming (U), hemorrhage (H), scoliosis (S), paralysis (P) and loss of control of melanophores (M). Of the 26 fish with grossly visible indications of injury 15 were examined histologically. Each row in the table indicates an individual fish and specific lesions are indicated by an X.

Species	Gross injury	Vertebral		Notochord hernia	Hemorrhage	Muscle necrosis
		Fract.[a]	Comp.[b]			
Bluegill						
Fish 1	H		X	X	X	
Fish 2	H		X		X	X
Fish 3	U		X			X
Largemouth bass	H				X	X
Channel catfish						
Fish 1[c]	H,S		X		X	X
Fish 2	H	X	X	X	X	X
Fish 3[c]	H		X	X	X	X
Fish 4[c]	H	X	X	X	X	X
Fish 5[c]	H		X	X	X	X
Striped Bass	H,S	X	X		X	X

(continued)

Table VII-3. Continued.

Species	Gross injury	Vertebral		Notochord hernia	Hemorrhage	Muscle necrosis
		Fract.[a]	Comp.[b]			
Nile tilapia						
Fish 1	U					X
Fish 2	U					X
Rainbow trout						
Fish 1	M		X	X		
Fish 2	P		X	X		X
Fish 3	P		X	X		X

[a]Fracture

[b]Compression and subluxation

[c]Killed by electroshock

Table VII-4. The number of electroshocked fish without grossly visible injuries but having lesions detected by histological examination. Control fish were also examined. Fish that were electroshocked were exposed for 20 s (5 and 20 s for bluegill) to 60-Hz PDC in water with 98-102 µS/cm ambient conductivity and temperature of 24-26°C. Largemouth bass were 15-35 mm TL, bluegill were 14-22 mm TL, and channel catfish were 16-39 mm TL. The lesions of individual fish with multiple lesions are indicated by an X.

Observation	*N*	Vertebral		Notochord hernia	Hemorrhage	Muscle necrosis
		Fract.[a]	Comp.[b]			
Control fish examined						
Bluegill	5	0	0	0	0	1
Largemouth bass	12	0	0	0	0	1
Channel catfish	4	0	0	0	0	0
Surviving fish examined						
Bluegill						
4 V/cm, 20 s	16	0	0	0	0	10
8 V/cm, 5 s	12	2	2	1	3	8
Fish 1		X	X		X	X
Fish 2		X	X	X	X	
Fish 3					X	X
Largemouth bass						
2 V/cm, 20 s	22	0	0	0	2	4

(continued)

Table VII-4. Continued.

Observation	N	Vertebral Fract.[a]	Vertebral Comp.[b]	Notochord hernia	Hemorrhage	Muscle necrosis
Channel catfish						
4 V/cm, 20 s	15	2	6	4	2	9
Fish 1			X	X		X
Fish 2		X	X	X	X	X
Fish 3		X	X	X	X	
Fish 4			X			
Fish 5			X	X		X
Fish 6			X			X
Dead fish examined						
Bluegill						
8 V/cm, 20 s	3	0	0	0	0	1
Largemouth bass						
4-8 V/cm, 20 s	6	0	0	0	0	4

(continued)

Table VII-4. Continued.

Observation	N	Vertebral Fract.[a]	Vertebral Comp.[b]	Notochord hernia	Hemorrhage	Muscle necrosis
Channel catfish						
4-8 V/cm, 20 s	7	2	3	2	0	5
Fish 1			X	X		X
Fish 2		X	X	X		X
Fish 3		X	X			X

[a]Fracture

[b]Compression/subluxation

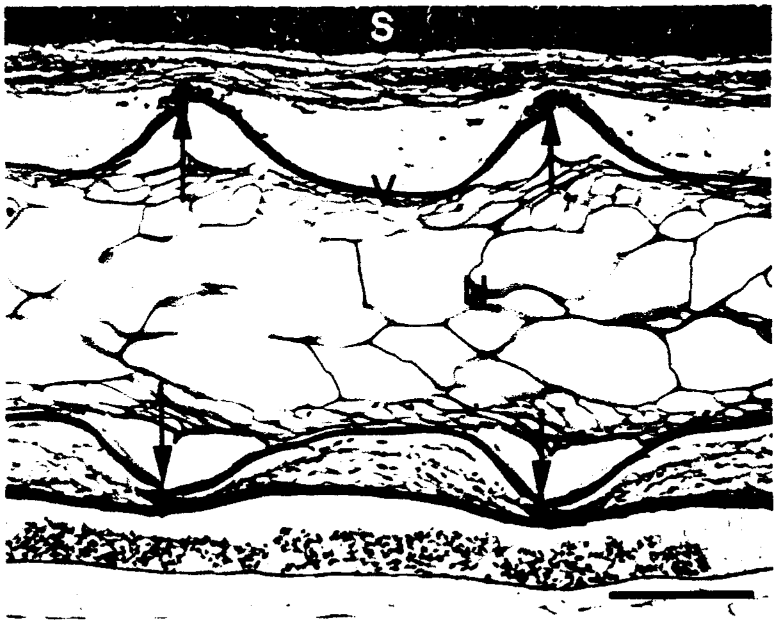


Figure VII-1. Sagittal section of the posterior trunk of a control channel catfish (25 mm TL) that was not electroshocked. Spinal cord (S) is dorsal to vertebrae (V); arrows indicate articulation between vertebrae. Notochord (N) is inside the vertebrae. Bar = 100 μm.

Figure VII-2. Sagittal section of the anterior tail of a bluegill (17.3 mm TL) that did not have gross indications of injury after electroshocking. Fracture (arrows) of a vertebra (V), hemorrhage (H). **a**, bar = 100 µm. **b**. Higher magnification of Figure VII-2 a. bar = 50 µm.

Figure 2a.

Figure 2b.

Figure VII-3. Sagittal section of posterior trunk of channel catfish (31.7 mm TL) that did not have gross injuries after electroshocking. Compression caused an outward bulge (B) near center of a vertebra (V), compacted the notochord (N), and caused subluxation of vertebrae (arrows). Bar = 100 µm.

Figure VII-4. Sagittal section of anterior tail of bluegill (17.9 mm TL) that did not have gross indications of injury after electroshocking. Vertebral compression with subluxation (arrows) of vertebrae (V). Bar = 100 µm.

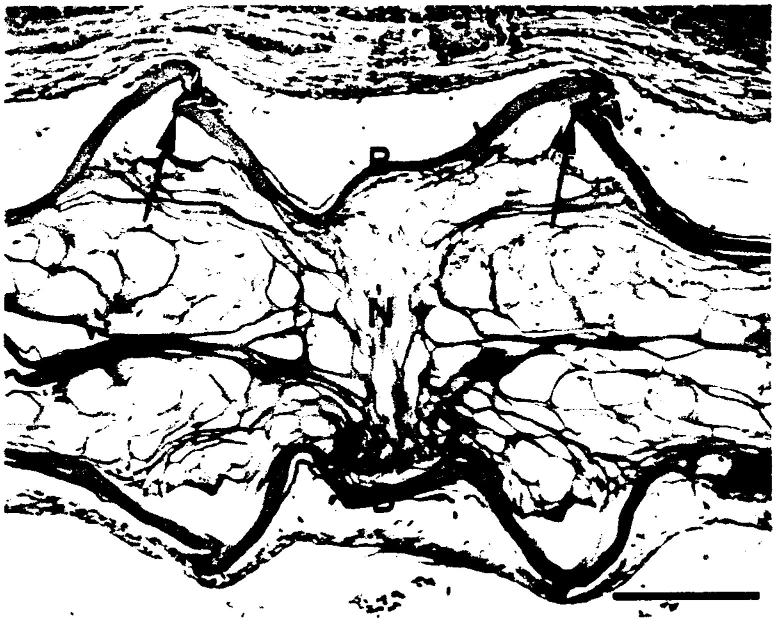


Figure 3.

Figure 4.

Figure VII-5. Sagittal section of anterior trunk of rainbow trout (79 mm TL) after electroshocking. Fish had paralysis of myomeres and survived during a 24-h observation after electroshocking. Herniation of the notochord (HN) between vertebrae (arrows) and into the spinal cord (S). Bar = 200 μm.

Figure VII-6. Sagittal section of mid trunk of a rainbow trout (36 mm TL) that had dark skin pigmentation posterior to the mid-trunk region after electroshocking. Ventral herniation of the notochord between adjacent vertebrae (V). The herniated notochord (HN) displaced trunk kidney. Renal tubule (R). **a.** Bar = 200 µm. **b.** Higher magnification of Figure VII-6a. Bar = 50 µm.

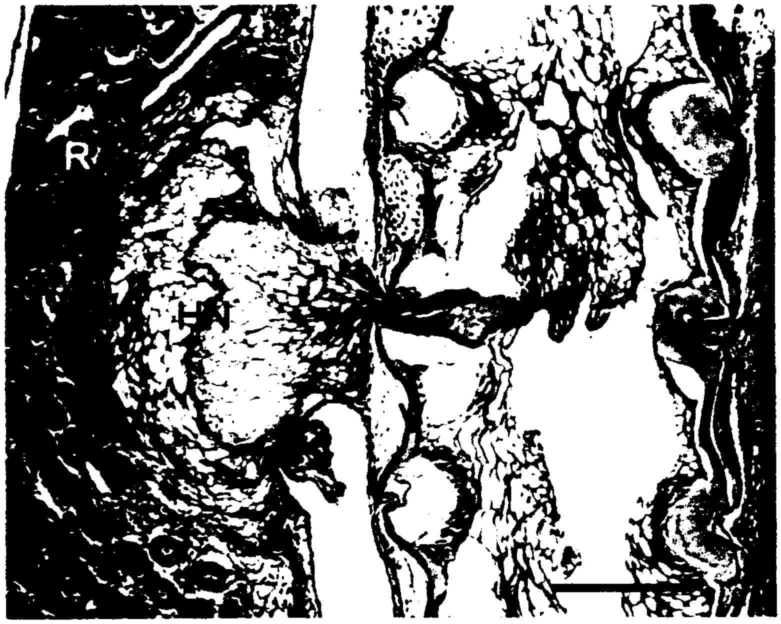


Figure 6a.

Figure 6b.

Figure VII-7. Sagittal section of anterior tail of channel catfish (23.4 mm TL) that did not have gross indications of injury after electroshocking. Herniation of the notochord (HN) into lateral skeletal muscle (M) with several necrotic muscle fibers (arrows) surrounded by normal appearing muscle fibers. Bar = 100 μm.

Figure VII-8. Sagittal section of brain of bluegill (15.6 mm TL) after electroshocking. Hemorrhage was grossly visible in the cranium and anterior tail. **a**. Hemorrhage (H) in the olfactory lobe. Bar = 50 μm. **b**. Hemorrhage (H) in the optic ventricle (O). Bar = 50 μm.

Figure 8a.

Figure 8b.

Figure VII-9. Frontal section of anterior tail of Nile tilapia (20.7 mm TL) that had uncoordinated swimming after electroshocking. **a**. Necrotic muscle fibers (arrows) surrounded by normal muscle. Bar = 100 µm. **b**. Higher magnification of Figure VII-9 a. Necrotic muscle fibers (arrows). Bar = 50 µm.

Figure 9a.

Figure 9b.

Figure VII-10. Sagittal section of anterior tail of bluegill (19.1 mm TL) after electroshocking. This fish had grossly visible hemorrhage in the anterior tail and survived for a 24-h observation after electroshocking. **a**. Necrotic muscle fibers (arrows) surrounded by normal muscle (M). Bar = 100 µm. **b**. Higher magnification of Figure VII-10 a. Necrotic muscle (NM) fibers, erythrocytes (E), and inflammatory cells (arrows). Bar = 20 µm.

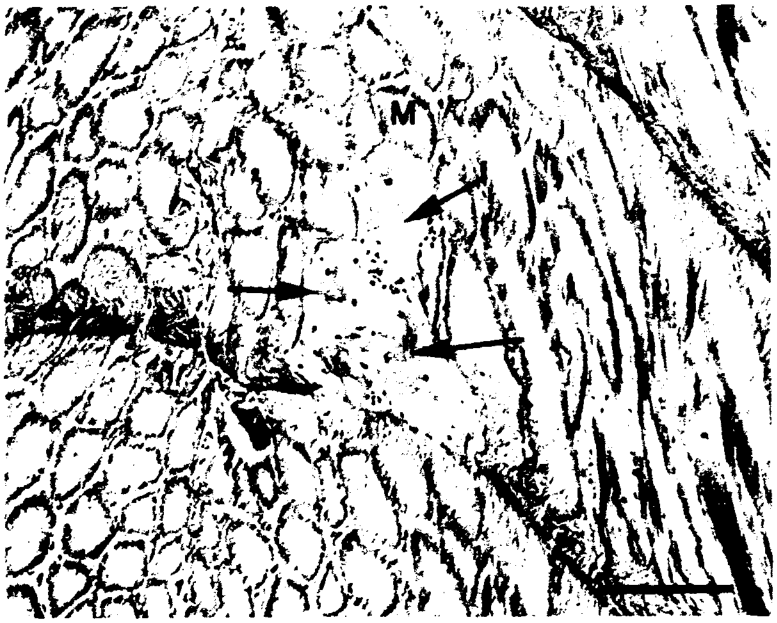


Figure 10a.

Figure 10b.

Figure VII-11. Sagittal section of a control largemouth bass (18 mm TL) that was not electroshocked. **a**. A necrotic skeletal muscle fiber (arrow) located in the adductor mandibulae muscle. Retina (R) of the eye is visible. Bar = 200 µm. **b**. Higher magnification of Figure VII-11 a. Coagulation and banding (arrows) of cytoplasm of necrotic muscle fiber. Normal muscle fibers (M) and epidermis (E). Bar = 50 µm.

Figure 11a.

Figure 11b.

Figure VII-12. Sagittal section of a control bluegill (15.4 mm TL) that was not electroshocked. **a.** Anterior tail myomeres with shrunken and condensed muscle fibers (arrows). Bar = 200 µm. **b.** Higher magnification of Figure VII-12 a. Coagulation and condensation of cytoplasm in necrotic muscle fibers (arrows) near the epidermis (E) and surrounded by normal muscle fibers (M). Bar = 50 µm.

Figure 12 a.

Figure 12 b.

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IX. APPENDIX

Table IX-1. Mean (SE in parentheses) values of blood plasma constituents of channel catfish (206-395 mm total length) 130-150 min after exposure to various electric fields (Chapter IV). Fish were obtained from ponds on the Auburn University Fisheries Research Station and were kept in 500-L laboratory tanks for 3 d before they were used in laboratory experiments. Fish were fed a dry diet (Zeigler Brothers, Gardners, Pennsylvannia), but were not fed within 24 h of experiments.

Hz	V/cm	*N*	Glucose mg/dL	TP[a] g/dL	Mg mg/dL	Ca mg/dL	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
0[b]	0[b]	10	73	3.8	3.1	11.9	136	4.8	117
			(5)	(0.1)	(0.1)	(0.2)	(1)	(0.2)	(1)
0[c]	0[c]	24	96	3.6	2.3	11.1	144	4.6	119
			(5)	(0.1)	(0.1)	(0)	(1)	(0.1)	(1)
	2	10	100	3.8	3.1	12.1	139	5.1	115
			(10)	(0.1)	(0.2)	(0.3)	(2)	(0.4)	(1)
7.5	4	9	87	3.7	2.8	12.4	145	4.8	120
			(9)	(0.1)	(0.1)	(0.4)	(6)	(0.1)	(2)

(continued)

Table IX-1. Continued.

Hz	V/cm	*N*	Glucose mg/dL	TP[a] g/dL	Mg mg/dL	Ca mg/dL	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
	8	12	103	4.0	2.8	11.6	141	5.6	113
			(6)	(0.1)	(0.2)	(0.5)	(2)	(0.3)	(3)
	2	10	86	3.8	2.8	11.5	140	4.5	115
			(6)	(0.1)	(0.2)	(0.4)	(2)	(0.2)	(1)
15	4	11	92	3.7	3.0	11.4	140	5.4	115
			(5)	(0.1)	(0.1)	(0.4)	(2)	(0.2)	(2)
	8	10	97	3.9	2.8	11.7	141	5.1	116
			(9)	(0.1)	(0.1)	(0.3)	(3)	(0.2)	(2)
	2	10	93	3.7	3.1	12.5	141	4.4	116
			(9)	(0.1)	(0.1)	(0.7)	(2)	(0.2)	(2)
30	4	10	83	3.7	3.1	12.0	141	4.9	117
			(4)	(0.1)	(0.1)	(0.2)	(2)	(0.2)	(2)
	8	9	77	3.7	3.1	11.5	139	4.2	115
			(4)	(0.1)	(0.1)	(0.4)	(2)	(0.1)	(2)
	2	10	96	3.9	2.8	12.7	146	4.6	121
			(6)	(0.1)	(0.1)	(0.3)	(4)	(0.2)	(3)

(continued)

Table IX-1. Continued.

Hz	V/cm	N	Glucose mg/dL	TP[a] g/dL	Mg mg/dL	Ca mg/dL	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
60	4	10	94	3.8	2.8	11.3	137	5.1	110
			(7)	(0.1)	(0.2)	(0.4)	(3)	(0.5)	(3)
	8	11	89	3.8	3.0	11.2	135	5.4	113
			(4)	(0.1)	(0.1)	(0.4)	(3)	(0.3)	(2)
	2	10	84	3.6	3.1	11.8	136	3.99	115
			(5)	(0.1)	(0.2)	(0.2)	(1)	(0.15)	(1)
120	4	9	93	4.0	2.9	12.2	146	4.53	123
			(6)	(0.2)	(0.1)	(0.2)	(4)	(0.17)	(4)
	8	11	95	3.7	2.7	11.2	138	4.98	116
			(7)	(0.1)	(0.1)	(0.4)	(1)	(0.55)	(2)
	2	10	81	3.5	2.8	11.7	135	4.75	114
			(3)	(0.2)	(0.1)	(0.3)	(1)	(0.26)	(1)
DC	4	10	86	3.7	2.9	11.7	133	4.28	112
			(6)	(0.1)	(0.1)	(0.3)	(1)	(0.19)	(1)
	8	10	85	3.7	2.9	11.7	137	4.16	116
			(9)	(0.1)	(0.1)	(0.3)	(3)	(0.12)	(2)

(continued)

Table IX-1. Continued.

Hz	V/cm	*N*	Glucose mg/dL	TP[a] g/dL	Mg mg/dL	Ca mg/dL	Na^+ mmol/L	K^+ mmol/L	Cl^- mmol/L
	2	11	94	3.7	2.7	12.4	141	5.19	116
			(9)	(0.1)	(0.1)	(0.3)	(2)	(0.34)	(2)
CPS	4	10	91	3.8	2.9	12.4	143	4.92	118
			(6)	(0.1)	(0.2)	(0.4)	(1)	(0.17)	(1)
	8	10	107	4.1	2.7	12.4	149	5.6	123
			(10)	(0.2)	(0.1)	(0.4)	(3)	(0.27)	(3)

[a]Total protein

[b]Control fish received the same handling as electroshocked fish but were not exposed to an electric field.

[c]Control fish sampled directly from the holding tank and only received handling associated with blood sampling.

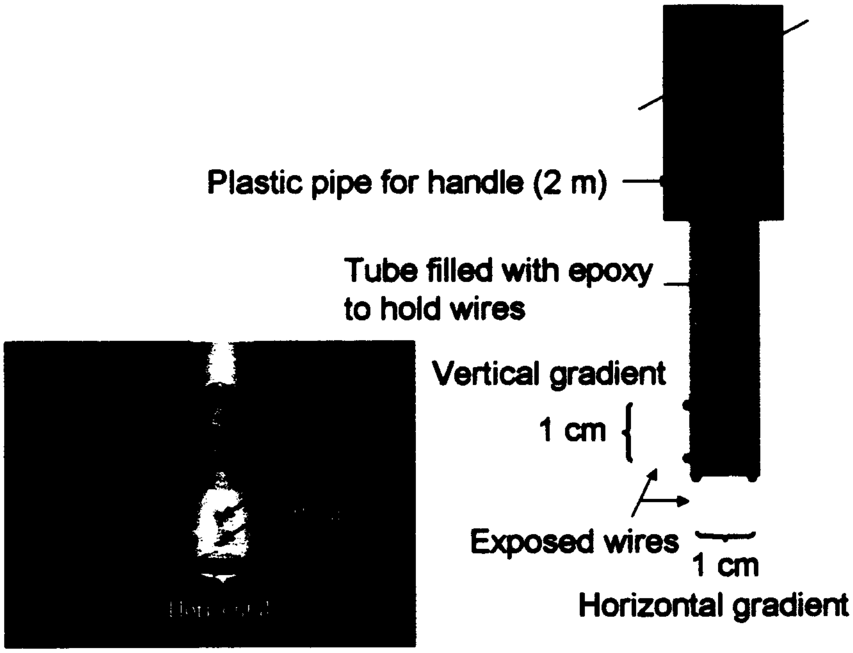

Figure IX-1. Voltage gradient measurement probe used to measure in-water voltage gradients around electrofishing boats (Chapter II). In the probe, copper wires were embedded in epoxy with their tips exposed and separated by 1 cm. A two-channel oscilloscope, with one channel connected to the vertical wires and the other channel to connected to the horizontal wires, was used to measure the voltage gradient of both vectors simultaneously.

Electrofishing Power Requirements in Relation to Duty Cycle

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Abstract.— Intensity and extensity of an electrofishing field depend on the amount of electrical power transmitted between electrodes. Pulsating the delivery of DC increases intensity by producing bursts of peak power, and extensity by expanding the radius of the threshold power needed to immobilize fish. Also, continuous DC and the various forms of pulsed DC stimulate the fish's nervous system differently, and differ in the power they require to immobilize fish. Under controlled laboratory conditions, we measured the electrical power needed to immobilize fish of various species and sizes with duty cycles (i.e., fraction of time a field is energized) ranging from 0.015 to 1. Test fish included eight species of various sizes. There was a significant inverse parabolic relation between power to achieve immobilization and duty cycle, with the least power required at duty cycles between 0.1 and 0.5. Operating within this range of duty cycles would allow an increase in the radius of action relative to higher duty cycles, while requiring the least power to immobilize fish. The power required to tetanize fish after 15 s was generally higher than that needed to immobilize them within 3 s, but the difference between these two power values increased with duty cycle. Electrofishing with high duty cycles can potentially reduce levels of injury by providing improved control over avoidance of tetany.

Electrofishing involves transmitting electricity from a power source through a metal electrode into the water and back into another electrode of opposite polarity. The intensity and extensity of the field created between the electrodes dictate electrofishing success. Electrofishing fields are heterogeneous as electrical energy radiates and spreads around and between electrodes, resulting in fields whose strength is greatest next to the electrodes and gradually dissipates as horizontal and vertical distance from the electrodes increases (Reynolds 1996). The actual field intensity encountered by a fish is determined by the extensity of the field and the fish's position within the field.

Intensity and extensity of an electrofishing field depend on the amount of electrical power that can be transmitted between electrodes, which in turn hinges on water conductivity, electrode shape, and power source (Novotny 1990; Kolz 1993). Moreover, intensity and extensity can be controlled by how the electricity is delivered through the field. Pulsating the delivery of DC increases intensity by producing large bursts of peak power through the aid of transformers and capacitors (Novotny 1990). Pulsating the delivery of DC also increases extensity of the field by expanding the radius of action, because the threshold power needed to immobilize fish radiates farther away from the electrodes. In addition to intensity and extensity of an electric field, how the power is presented (i.e., continuous DC or the various forms of pulsed DC) affects how a fish's nervous system is stimulated (Lamarque 1990; Sharber and Black 1999), and possibly the power required for immobilization.

A diversity of pulse trains may be delivered by manipulating pulse period (duration of time on) and pulse frequency (incidence of time on). Duty cycle indicates the fraction of time the electrofishing field is activated. Under controlled laboratory conditions, we measured the electrical power needed to immobilize fish of various species and sizes with a selection of pulse frequencies, pulse periods, and therefore duty cycles. The objective of this analysis was to identify duty cycles that required the least power to immobilize fish. Such settings would tend to be most efficient for electrofishing, because they would allow the maximum radius of action with the minimum amount of power.

Methods

Test Equipment

All testing was conducted indoors in a polyethylene tank 2.0 m long, 1.5 m wide, and 1.0 m deep. The tank was filled to a depth of 10 cm with well water. The cross sectional profile of the tank was faced with two, 1.6-cm thick aluminum plate electrodes positioned 65 cm apart, perpendicular to the longitudinal axis of the tank. Electricity was supplied to the plates via a Smith-Root 15-D POW unit (Smith-Root, Inc., Washington) modified to allow continuous rather than discrete voltage control, and equipped with additional smoothing capacitors to eliminate spikes and reduce ripples at the peak of pulses (i.e., ripples averaged $\pm$ 6% of the amplitude). Conditions within the tank produced a homogeneous electrical field with a constant voltage gradient. Specific conductivity ($C_s, \mu S \cdot cm^{-1}$) and ambient water temperature (T_w) were recorded with a YSI 30/10 FT meter (Yellow Springs Instruments, Ohio). The meter read C_s at specific temperature (T_s, 25° C). Ambient water conductivity (C_w) was estimated from specific conductivity, specific temperature, and ambient water temperature (Reynolds 1996) as

$$C_w = C_s \cdot 1.02^{T_w - T_s} \qquad (1)$$

Electrical Treatments

Ten electrical treatments representative of pulse frequencies and periods commonly available in commercial units were considered (Table 1). Peak voltage (V_{pk}), frequency, and period were measured within the energized field with a Tektronix THS720A oscilloscope (Tektronix, Inc., Oregon). Following Kolz and Reynolds (1989), V_{pk} was used to calculate power density (P_w) as

$$P_w = C_w \cdot \left(\frac{V_{pk}}{d} \right)^2 \qquad (2)$$

where d is the distance between the electrodes (i.e., 65 cm). Duty cycle was computed as the product of pulse period (ms) and pulse frequency (Hz) divided by 1,000.

We applied the ten electrical treatments to various sizes of eight species selected because they were readily available from local fish culture facilities and streams. However, limited fish availability did not allow application of all electrical treatments to a balanced combination of species and sizes (Table 1). Prior to testing, fish were seined from culture ponds or from streams, held in concrete raceways or polyethylene circular tanks for at least two weeks, and maintained in good condition on a diet of live or prepared food, depending on the species. During testing, fish were transferred one at a time to the test tank and confined in the area between the two electrodes. After allowing 3-10 s for the fish to orient, and when the fish was positioned perpendicular to the electrodes, the current was switched on for 15 s. As individuals, fish were treated only once and to a single voltage, but as a group, fish were exposed to voltages incrementing from near zero to the highest levels allowed by our equipment. Two immobilization thresholds were recorded: (1) immobilization status at 3 s, recorded as 0 for no immobilization and 1 for immobilization; and (2) immobilization status at 15 s, recorded as 0 for narcosis (fish immobilized, muscles relaxed, still breathing) and 1 for tetanus (fish immobilized, muscles rigid, and no breathing motions). The 3 s period estimated the time within which if the fish was not immobilized, it would likely escape the electrical field; the 15 s period estimated the maximum amount of time that a fish would be exposed to electricity in an actual field setting. As many as 18-35 fish were used per treatment, depending on ease of identifying the immobilization threshold. The reactions of each fish were observed and written down, but were also recorded via a video camera positioned over the tank, allowing review of responses to verify the accuracy of live observations.

Immobilization Thresholds

Field strength has traditionally been measured as voltage gradient, current density, or power density (voltage gradient X current density). More recently, Kolz (1989) suggested that the success of electrofishing depends on the fraction of the power density that is transferred to the fish. The power transfer model has been shown to reduce variability of survey data (Burkhardt and Gutreuter 1995) and to adequately predict power levels required to elicit immobilization of fish over a wide range of water conductivities (Kolz and Reynolds 1989; Miranda and Dolan, in review).

For each electrical treatment, the dependent binary immobilization response y_i recorded for each fish was regressed on the independent variable P_w applied to each fish, using the logistic regression model

$$y_i = \beta_o + \beta_i S + \beta_1 \log_e P_w \quad (3)$$

where β_o represents the intercept parameter, $\beta_i S$ the differential effect attributed to the species-size category, and β_1 the slope parameter for $\log_e P_w$. The resulting logistic model was used to estimate the peak power density threshold required for a 0.95 probability of immobilizing (at 3 s) or tetanizing (at 15 s) the fish ($P_{w,\,0.95}$), and $P_{w,\,0.95}$ was used to estimate the power transferred into the fish ($P_{f,\,0.95}$) as (Kolz 1989)

$$P_{f,0.95} = P_{w,0.95}\left(4\frac{C_f}{C_w}\right)\left(1+\frac{C_f}{C_w}\right)^{-2} \quad (4)$$

where C_f is the estimated standard conductivity of fish (115 $S\ c\ m^{-1}$) suggested by Miranda and Dolan (in review). Equations 3 and 4 were applied separately to the values describing immobilization status at 3 s and 15 s.

Effect of Duty Cycle

The effects of pulse period and frequency on power required to immobilize fish were examined by plotting $P_{f,\,0.95}$ on duty cycle. To account for potential differences in fish species and size that affect power requirements, fish species (S) and volume (V) were included in a model designed to assess the effect of duty cycle (D)

$$\log_{10} P_{f,\,0.95} = \beta_o + \beta_1 \log_{10} D_i + \beta_2 \log_{10} D^2_i + \beta_3 \log_{10} V_j + \beta_k S \quad (5)$$

where β_o represented the model's intercept parameter, β_1 and β_2 the slope parameters for the effect of the i^{th} duty cycle, β_3 the slope parameter for fish volume, and β_k the effect of the k^{th} species. $P_{f,\,0.95}$ represented either power transferred into the fish to immobilize within 3 s, or to tetanize after 15 s. We used fish volume to index size because we had previously identified it as the size descriptor best related to the level of electric power required for immobilizing fish (Dolan and Miranda, in review). Interactions among main effects were also examined. Adequacy of the models was judged by the magnitude of the R^2 value, and by inspecting residual plots.

Results

In all, 1,578 fish were included in these tests, ranging in mean total length from 53 to 328 mm (overall mean = 159), and volumes from 2 to 336 cm^3 (overall mean = 103). Water temperatures at which fish were held and tested ranged from 17 to 27°C (mean = 23°C). Specific conductivity was relatively invariable at 195±4 $S\ cm^{-1}$ throughout the study. However, due to fluctuations in ambient water temperature, ambient water conductivity (equation 1) ranged from 176 to 201 S c m^{-1}. Peak voltages applied in these water conditions ranged from 12 to 1,100 V, and peak power densities ranged from 7 to 147,500 W cm^{-3}.

To immobilize fish within 3 s, estimates of $P_{f,\,0.95}$ ranged from over 88,000 W cm^{-3} for immobilization of the small-bodied *Etheosthoma whipplei* with 0.015 duty cycle, to under 50 W cm^{-3} for large-bodied fish of several species treated with 0.11-0.66 duty cycle (Figure 1). For immobilization at 3 s and tetany at 15 s, there was a significant inverse parabolic relation between $P_{f,\,0.95}$ and duty cycle (Table 2), indicating power requirements were lowest at an intermediate duty cycle between 0.1 and 0.5. The models for the 3 s and 15 s data identified no significant species effect, but a significant effect of fish volume, suggesting that any species differences were potentially overshadowed by the effect of fish size. The interaction between fish volume and duty cycle was marginally significant for the 3-s model (P = 0.14) and not significant for the 15-s model (P = 0.71); thus, they were excluded from the final models.

The coefficient of determinations (R^2 = 0.78 and 0.72) indicated the models adequately described the effect of duty cycle on power requirements while accounting for fish size. Nevertheless, for both models, a plot of the residuals against duty cycle and volume revealed a possible lack of fit, wherein residuals for PDC15-4 and PDC15-6 tended to be higher than zero and residuals for PDC60-1 tended to be less than zero. This lack of fit would cause the models to overestimate the power requirements with PDC60-1, and underestimate the power requirements with PDC15-4 and PDC15-6.

The $P_{f,\,0.95}$ required to tetanize fish after 15 s was generally higher than that needed to immobilize them within 3 s. Nevertheless, the margin of difference between the two power values changed relative to duty cycle. For some low duty cycle treatments, fish could not be immobilized within 3 s without tetanizing them by the end of the 15 s period. Conversely, for high duty cycle treatments there was a wider margin of power requirements between immobilization and tetany, and thus fish immobilized within 3 s remained only narcotized by the end of the 15 s period. This effect is illustrated by a plot of the two models (Figure 2).

Discussion

Although our tank experiments were able to control for many sources of error commonly associated with field electrofishing, some estimation errors could not be avoided. While we strived to maintain ambient conditions as constant as practicable, variability in water temperature had to be accepted owing to the seasonal availability of test fish. Error could have been introduced by the 10°C range of experimental temperatures, which possibly influenced fish conductivity and reaction thresholds (Whitney and Pierce 1957). Furthermore, identification of the immobilization threshold relied on an observer's ability to discern the moment fish were immobilized. As duty cycle decreased, fish exhibited a vigorous forced swimming behavior that sometimes made it hard to assert whether the fish had been immobilized within 3 s, even after reviewing recorded videos. Despite these inaccuracies, error around our explanatory model, which included both experimental error as well as model lack of fit, was relatively small.

The lack of fit appeared to be contributed mainly by the pulsed DC 15 Hz, 4 ms and pulsed DC 15 Hz, 6 ms treatments. Residual analyses showed that the power needed to immobilize fish with these low pulse frequencies was greater than that required by higher frequency treatments of similar duty cycles. This discrepancy suggests that immobilization response is not fully accounted for by duty cycle, but is also affected by a potential interaction between frequency and period. We were unable to further examine this effect because of the unbalanced nature of our treatment combinations, and because the continuous DC treatment cannot be described in terms of frequency or period (Table 1). Nevertheless, this lack of fit was trivial in the context of our conclusions about duty cycles that maximize radius of action and minimize power requirements.

In addition to the effect of duty cycle, our descriptive models identified a lack of species effect and a strong size effect. The absence of a species effect possibly reflects the overwhelming importance of fish size. Dolan and Miranda (in review) suggested that while some species differences could be expected due to differences in fish conductivity, most of the variability in immobilization response is attributed to fish size.

Power densities needed to immobilize fish within 3 s and tetanize them within 15 s decreased with increases in fish size and duty cycle, but increased rapidly at duty cycles lower than about 0.1. Similarly, Lamarque (1967) reported that varying the period of a fixed 100 Hz waveform resulted in an increase in the threshold of anodic taxis only when duty cycles were less than 0.1. Novotny and Priegel (1974) indicated that 0.25 and 0.5 duty cycles produced similar results, and that a 0.1 duty cycle was less effective. Kolz and Reynolds (1989) reported a decrease in the power density required to immobilize 6-9 cm goldfish *Carassius auratus* as they varied duty cycle from 1 to 0.1, but did not consider duty cycles lower than 0.1. Given our results and those reported by other authors, it appears that efficiency of electrofishing can be maximized by using duty cycles between 0.1 and 0.5. Such strategy would allow an increase in the radius of action by operating with duty cycles that allow more power to be transmitted through the electrical field while at the same time requiring less power to immobilize fish. The increase in the radius of action is a result of pulsating the delivery of DC, which increases its intensity by producing large bursts of power that radiate with more strength farther away from the electrodes (Novotny 1990; Reynolds 1996). The decreased power requirement at intermediate duty cycles reflects changes in response to different electrical stimuli by the fish's nervous system, but the mechanisms are not well understood and currently being debated in the literature (Lamarque 1990; Sharber et al. 1995; Sharber and Black 1999).

Moreover, stream electrofishing equipment sometimes relies on battery-powered electrofishers. Because battery power is limited and battery life is finite, use of intermediate duty cycles would facilitate backpack electrofishing by increasing the time between battery charge or replacement. Beaumont et al. (2000) reported that battery longevity in their backpack equipment was extended ten fold by reducing the period of pulsed DC 60 Hz from 6 ms (duty cycle = 0.36) to 0.5 ms (duty cycle = 0.03), and extended three fold when a gated burst with 30 Hz and 0.9 ms period (duty cycle = 0.027) was applied.

Although power requirements and capture efficiency are important considerations in selecting waveforms for electrofishing, increased taxis (attraction towards the anode, i.e., positive electrode) and thrashing can influence the choice of waveform. Reynolds (1996) commented that continuous DC can induce taxis given appropriate thresholds were reached, but that the taxis responses to pulsed DC were less predictable. In our tests, we noted that the continuous DC treatment caused fish to exhibit forced swimming towards the anode before being immobilized within 3 s. This attraction occurred immediately upon electrification of the field, was highly conspicuous in some species but occurred in all species and sizes treated with continuous DC. However, at high levels of DC fish were immobilized instantly once the field was electrified, with no obvious forced swimming towards the anode. Attraction towards the anode was observed in a few fish treated with pulsed DC, but was not as striking as with continuous DC. The high power levels required for immobilizing fish with low duty cycles tended to encourage forced swimming and thrashing rather than immobilization. This observation is consistent with those made by Corcoran (1979) and Gilliland (1987) who reported that low duty cycles made ictalurids easier to detect because of thrashing, but that collection often required a chase boat because fish were not immobilized.

A central finding of our study was the changing margin of difference between the amount of electrical power required to tetanize fish after 15 s and that required to immobilize them within 3 s. At high duty cycles, there was a large margin of difference whereas at low duty cycles the margin of difference decreased, to the extent that the power needed to immobilize fish within 3 s would inevitably produce tetany at 15 s. At high duty cycle settings, power could be applied in a way that it immobilized fish within 3 s and produced only narcosis at 15 s, allowing the fish to resume swimming when power was deactivated. Lower levels of injury have been reported for fish that are narcotized rather than tetanized by electrofishing (Lamarque 1990; Reynolds 1996). Thus, electrofishing with high duty cycles can potentially reduce levels of injury by providing improved control over avoidance of tetany.

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Table 1. Electrical treatments, duty cycles, species, and sizes included in this evaluation. The total volume values correspond to the order of the listing of total lengths.

Treatment	Pulse frequency (Hz)	Pulse period (ms)	Duty cycle	Test species	Total length (mm)	Total volume (cm^3)
DC	None[a]	None[a]	1.000	*Ictalurus punctatus*	56, 163, 311, 313, 317	4, 31, 303, 312, 318
				Lepomis macrochirus	67, 159	12, 109
				Micropterus salmoides	73, 221	6, 189
				Morone hybrid	180	101
				Pomoxis nigromaculatus	155	83
PDC110-6	110	6	0.660	*Ictalurus punctatus*	69, 160, 311	5, 31, 309
				Lepomis macrochirus	68, 156	12, 104
				Micropterus salmoides	75, 220	6, 186
				Morone hybrid	175	96
				Pimephales notatus	55	2
				Pomoxis nigromaculatus	142	70
PDC110-1	110	1	0.110	*Ictalurus punctatus*	57, 166, 310, 327	4, 32, 303, 336
				Lepomis macrochirus	68, 168	12, 118
				Micropterus salmoides	72, 207	6, 166
				Morone hybrid	174	96
				Pimephales notatus	57	2
				Pomoxis nigromaculatus	151	77
PDC60-6	60	6	0.360	*Ictalurus punctatus*	158	31
				Lepomis macrochirus	67	12
				Micropterus salmoides	62	4
PDC60-1	60	1	0.060	*Ictalurus punctatus*	161, 310, 312	31, 303, 307
				Lepomis macrochirus	69	12
				Micropterus salmoides	62	4
				Pomoxis nigromaculatus	155	83
PDC30-1	30	1	0.030	*Ictalurus punctatus*	312	306
PDC20-1	20	1	0.020	*Ictalurus punctatus*	310	303
PDC15-6	15	6	0.090	*Ictalurus punctatus*	63, 159, 313	4, 30, 310
				Lepomis macrochirus	68, 148	12, 93
				Micropterus salmoides	75, 222	6, 197
				Morone hybrid	176	100
				Pimephales notatus	61	2
				Pomoxis nigromaculatus	159	86
				Semotilus atromaculatus	63	4
PDC15-4	15	4	0.060	*Pomoxis nigromaculatus*	158	87
PDC15-1	15	1	0.015	*Etheostoma whipplei*	53	3
				Ictalurus punctatus	67, 164, 311, 328	5, 31, 305, 330
				Lepomis macrochirus	68, 157	12, 104
				Micropterus salmoides	75, 215	6, 189
				Pomoxis nigromaculatus	157	85
				Semotilus atromaculatus	62	4

[a] DC is on continuously.

Table 2. Regression models descriptive of the power that must be transferred into the study fish ($P_{f,\,0.95}$) to immobilize within 3 s or tetanize by the end of 15 s. The parameters correspond to the model $\log_{10} P_{f,\,0.95} = \beta_o + \beta_1 \log_{10} D_i + \beta_2 \log_{10} D^2_i + \beta_3 \log_{10} V_j$ where D = duty cycle and V = fish volume (cm^3). All parameters were significantly different from zero at $P \leq 0.01$. Standard errors are given in parentheses.

Parameter	Immobilization status at	
	3 s	15 s
β_o	3.241 (0.156)	3.844 (0.173)
β_1	1.426 (0.268)	1.820 (0.301)
β_2	1.265 (0.146)	1.268 (0.153)
β_3	-0.513 (0.071)	-0.428 (0.106)
R^2	0.78	0.72

Figure Captions

Figure 1. Relationship between power transferred to immobilize 95% of fish within 3 s ($P_{f\ 0.95}$) and duty cycle. Differences in circle sizes denote relative differences in $\log_{10}$ of fish volume. The labels next to the dashed vertical lines identify the treatments listed in Table 1 and their corresponding duty cycles. The dashed curve denotes $P_{f\ 0.95}$ in relation to D at a $V = 100\ \text{cm}^3$.

Figure 2. Relationship between duty cycle and power transferred ($P_{f\ 0.95}$) required for immobilizing fish within 3 s or tetanizing them at 15 s. Curves were derived with the equations in Table 2 for $D = 0.015\text{-}1$ and $V = 10$ and $300\ \text{cm}^3$. The figure illustrates how for fish of a given size the margin of difference in power needed to immobilize fish within 3 s or tetanize them at 15 s increases directly with duty cycle.

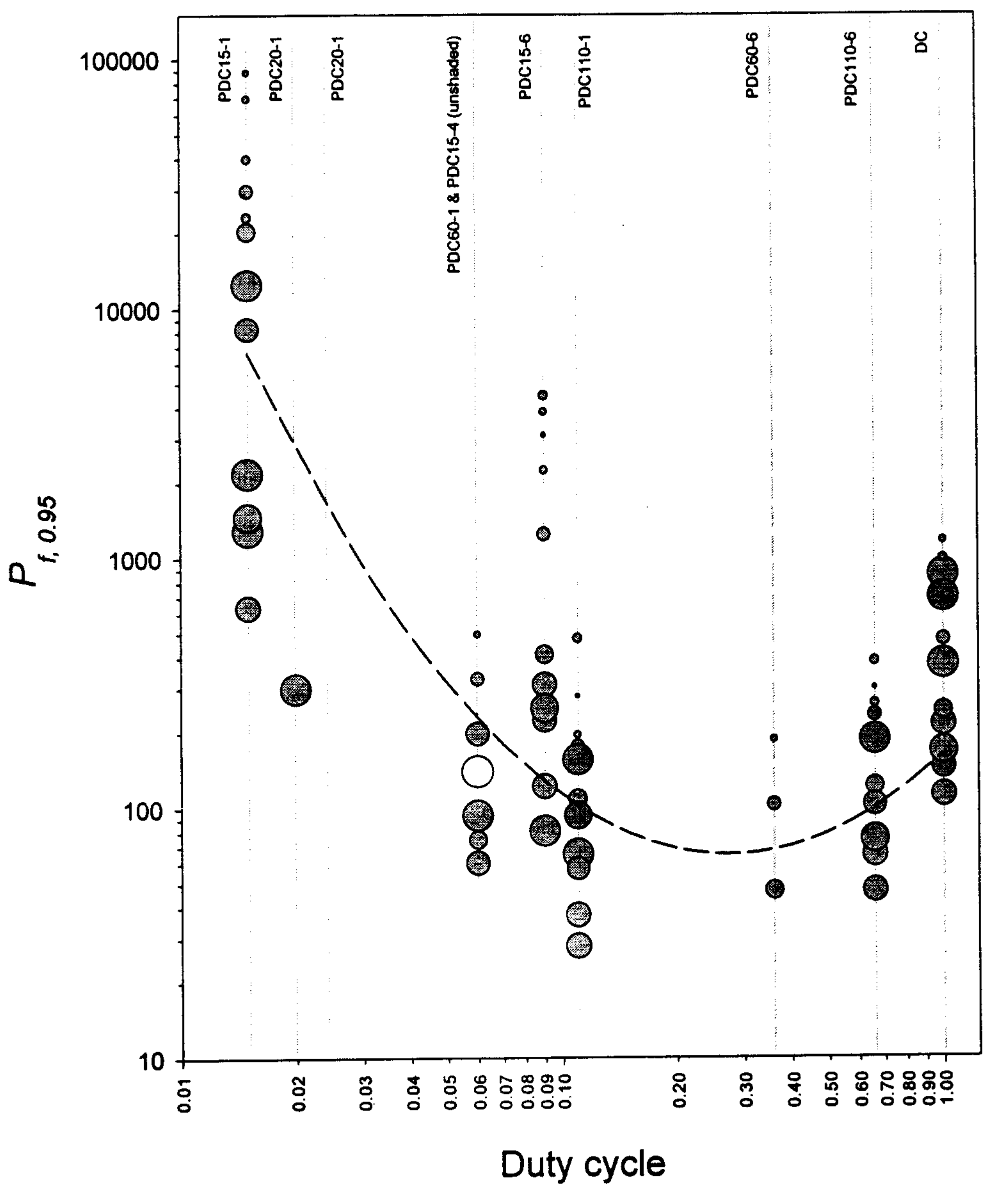
PDC15-1
PDC20-1
PDC20-1
PDC60-1 & PDC15-4 (unshaded)
PDC15-6
PDC110-1
PDC60-6
PDC110-6
DC
100000
10000
1000
100
10
$P_{f,\,0.95}$
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0.20
0.30
0.40
0.50
0.60
0.70
0.80
0.90
1.00
Duty cycle

Figure 1.

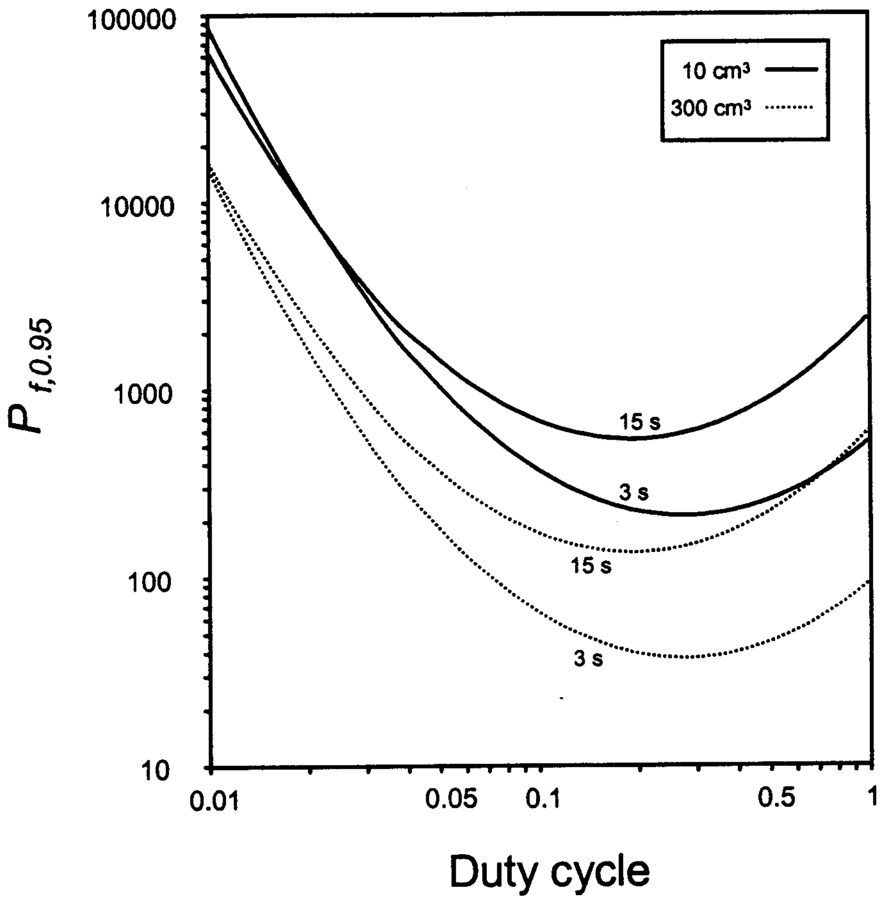
100000
10000
1000
100
10
P f,0.95
0.01
0.05
0.1
0.5
1
Duty cycle
10 cm³
300 cm³
15 s
3 s
15 s
3 s

Figure 2.

Electrofishing Injury and Mortality of Warmwater Fishes is Influenced by Species, Size, and Duty Cycle

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Abstract.- Most studies of injury associated with electrofishing have focused on salmonid fishes, but few have given attention to warmwater fishes. Under controlled laboratory conditions, we treated bluegill *Lepomis macrochirus*, channel catfish *Ictalurus punctatus*, and largemouth bass *Micropterus salmoides* of various sizes to duty cycles ranging from 0.015 to 1. At each duty cycle, fish were exposed to power densities in excess of immobilization thresholds to determine the incidence of hemorrhage, spinal injury, and mortality. For fish treated with power densities high enough to immobilize them within 3 s, incidence of hemorrhage averaged 3% (range 0-25), spinal injury 3% (range 0-22%, and mortality 10% (range 0-75). Injury and mortality were dependent on duty cycle, fish size, and species, and in most cases, interaction among these variables. Fish that were tetanized by the electrical treatment were more likely to suffer injury and mortality than fish that were only narcotized. Electrofishing with high duty cycles can reduce electrofishing-induced injury and mortality to warmwater fish. Power output should be managed to induce narcosis and avoid tetany.

Electrofishing is a widely used, accepted, and effective method for collecting freshwater fishes (Simpson and Reynolds 1977; McMichael et al. 1998; Vaux et al. 2000). Historically, studies have shown that exposure of fish to electric current can lead to injury, particularly tissue hemorrhage (i.e., bleeding of blood vessels and capillaries) and spinal injury (i.e., compression, misalignment, or fracture of the vertebral column), and can even cause immediate or delayed mortality (Hauck 1949; Spencer 1967; Sharber and Carothers 1988). Injured fish do not always suffer long-term physical handicap or die because injuries often heal (Horak and Klein 1967; Hudy 1985; Schill and Elle 2000). However, survival may be indirectly influenced by the adverse effects of electric shock on behavior, health, growth, and reproduction (Gatz and Adams 1987; Mesa and Schreck 1989; Muth and Ruppert 1996). These adverse effects have prompted claims that studies of fish populations might be seriously compromised by the use of electrofishing (e.g., Bardygula-Nonn 1995). For example, failing to minimize electrofishing-induced injury and mortality in fish collected for mark-recapture studies may lead to inflated population estimates or deflated exploitation estimates (Pratt 1955; Barrett and Grossman 1988). Moreover, the detrimental effects of electrofishing may severely impact threatened or endangered fish populations (Barrett and Grossman 1988; Ruppert and Muth 1997). Due to concerns regarding electrofishing injury and mortality, some researchers have suggested that electrofishing techniques and theory require further examination (e.g., Reynolds 1996).

The severity of injury and potential lethality that can result from electroshock varies with fish species. Differences in morphology, physiology, and behavior often lead to different degrees of vulnerability. Tissue resistance in bony fish species varies greatly, and cartilaginous fishes are less conductive of electrical current than bony fishes (Reynolds 1996). Vestigial-scaled (e.g., ictalurids) and fine-scaled (e.g., salmonids) fishes are reportedly more susceptible to electroshock than coarse-scaled fishes (e.g., cyprinids; Reynolds 1996). Species differences were readily apparent in the study conducted by Thompson et al. (1997) who found differences in rate of injury between brown trout *Salmo trutta* and rainbow trout *Oncorhynchus mykiss*. Also, Kocovsky et al. (1997) determined that salmonid populations in Colorado streams remained stable after eight years of electrofishing, while the abundance of longnose suckers *Catostomus*

catostomus inhabiting the same streams decreased, suggesting a possible effect of electrofishing on survival. However, Pratt (1955) found no evidence to suggest differences in susceptibility to mortality of rainbow, brown, and brook trout *Salvelinus fontinalis*.

Body size is also important in determining vulnerability of fish to capture, injury, and mortality (Dolan and Miranda, in review). Early studies focused on the relation of size to capture efficiency (e.g., Shetter 1947; Holmes 1948; Smith and Elson 1950), but only a few studies considered the effect of size on mortality (e.g., Hauck 1949; Rayner 1949; Pratt 1955). It was not until the 1980s and 1990s that researchers began to focus more closely on the effect of fish size on incidence of injury and mortality. For instance, Hudy (1985) suggested that differences in injury and mortality between rainbow and brook trout exposed to electrofishing might be attributed to size effects rather than species differences. In addition, Hollender and Carline (1994) found that incidence of injury in wild brook trout increased with fish length, and Bardygula-Nonn et al. (1995) observed higher mortality in small centrarchids than large ones.

The way in which electrical power is presented (i.e., continuous DC or the various forms of pulsed DC) affects how a fish's nervous system is stimulated (Lamarque 1990; Sharber and Black 1999), and possibly the incidence of injury and mortality. Continuous DC is often regarded as the least injurious waveform (Lamarque 1990; Reynolds 1996). Fish mortality rarely occurs because of DC electroshock, but physical injuries and physiological trauma have been noted, although often undetectable externally (Sharber and Carothers 1994). Pulsating the delivery of DC can potentially increase intensity by producing large bursts of peak power through the aid of transformers and capacitors (Novotny 1990). Because of increased intensity, pulsed DC can produce effects that are more severe than continuous DC (Reynolds 1996).

Pulsed DC waveforms are composed of a pulse frequency (incidence of time on) and pulse period (duration of time on). Both variables can be manipulated to produce a variety of pulse trains. Duty cycle indicates the fraction of time that the electrical field is energized (i.e., pulse frequency x pulse period/1,000 ms). Furthermore, pulse frequency and pulse period (thus, duty cycle) are thought to affect incidence of injury and mortality. Vibert (1967) recommended that fish should be collected with low-frequency settings to minimize injury. Sharber and Carothers (1994) findings supported Vibert's recommendation, as they showed that incidence of spinal injury to rainbow trout increased with increases in pulse frequency from 15 to 512 Hz. Moreover, research conducted by Dolan et al. (in press) suggested that incidence of hemorrhage in black crappie *Pomoxis nigromaculatus* was similarly related to pulse frequency. Sharber et al. (1994) recognized the potential for incidence of injury to be significantly reduced by employing low-energy, low-frequency, pulsed DC waveforms. Nevertheless, Lamarque (1990) indicated that electrical settings consisting of short-pulse durations were the most injurious to fish.

It has been hypothesized that injury may result from severe muscle contractions during tetany (Lamarque 1990). Tetany (fish immobilized, muscles rigid, and no breathing motions) is the last stage in a series of three general behavioral responses recognized in fish exposed to electroshock. It is preceded by narcosis (fish immobilized, muscles relaxed, still breathing), and fright (sporadic swimming). Many researchers (e.g., Vibert 1967; Lamarque 1990; Reynolds 1996) have suggested that injuries can be avoided if electrofishing equipment is operated at voltages strong enough to induce narcosis but not tetany.

Most injury and mortality experiments have been conducted on salmonid species. Little is known about the effect of continuous DC and pulsed DC settings on warmwater fishes. This lack of information led Reynolds (1996) to suggest that biologists actively pursue injury and mortality evaluations of non-salmonids. The objective of this study was to identify duty cycles that minimize risk of injury and mortality to selected warmwater fishes.

Methods

Test Tank and Power Source

All testing was conducted from March to December 1999 under controlled conditions at the Mississippi State University Aquaculture Center, where a laboratory was assembled and maintained. Experimentation was performed in a polyethylene tank measuring 2.0 m long, 1.5 m wide, and 1.0 m deep. The tank was filled to a depth of 10 cm with well water. The cross sectional profile of the tank was equipped with two, 1.6

cm thick aluminum plate electrodes positioned 65 cm apart, perpendicular to the longitudinal axis of the tank. Electricity for most treatments was supplied to the tanks via a Smith-Root 15-D POW electrofisher (Smith-Root, Inc., Washington) modified to allow continuous rather than discrete voltage control, and equipped with additional smoothing capacitors to eliminate spikes and reduce ripples at the peak of pulses (i.e., ripples averaged ± 6% of the amplitude). A Coffelt Mark X electrofisher (Coffelt Manufacturing, Arizona) was used to apply a Coffelt trademark pulse train (see below). Conditions within the tank produced a homogeneous electrical field with a constant voltage gradient. Specific conductivity (C_s, $\mu S/cm^{-1}$) and ambient water temperature (T_w) were recorded with a YSI 30/10 FT meter (Yellow Springs Instruments, Ohio). The meter read C_s at specific temperature (T_s, 25° C). Ambient water conductivity (C_w) was estimated from specific conductivity, specific temperature, and ambient water temperature as (Reynolds 1996) as

$$C_w = \frac{C_s}{1.02^{T_s - T_w}} \qquad (1)$$

Electrical Treatments

Eight electrical treatments representative of pulse frequencies and periods commonly available in commercial electrofishing units were considered. These included continuous DC (duty cycle = 1), pulsed DC 110 Hz, 6 ms (duty cycle = 0.66), pulsed DC 110 Hz, 1 ms (duty cycle = 0.11), pulsed DC 60 Hz, 6 ms (duty cycle = 0.36), pulsed DC 60 Hz, 1 ms (duty cycle = 0.06), pulsed DC 15 Hz, 6 ms (duty cycle = 0.09), pulsed DC 15 Hz, 1 ms (duty cycle =0.015), and Coffelt's CPS™ (duty cycle = 0.12). Coffelt's CPS™ (Complex Pulse System) was evaluated because the manufacturer claims that this system reduces myoclonic jerks and trauma, by merging high-pulse frequency with a low-frequency pattern. The CPS™ delivers a fixed complex pulse pattern consisting of three 240 Hz, 2.6 ms square-pulses, each separated by 1.6 ms, and repeated 15 times/second.

Peak voltage (V_{pk}), pulse frequency, and pulse period (i.e., width) were measured within the energized field with a Tektronix THS720A oscilloscope (Tektronix, Inc., Oregon). Following Kolz and Reynolds (1989), V_{pk} was used to calculate power density (P_w) as

$$P_w = C_w \cdot \left(\frac{V_{pk}}{d} \right)^2 \tag{2}$$

where d is the distance between the electrodes (i.e., 65 cm).

Test Fish

We applied the eight electrical treatments to bluegill *Lepomis macrochirus*, channel catfish *Ictalurus punctatus*, and largemouth bass *Micropterus salmoides* of various sizes. However, limited fish availability did not allow application of all electrical treatments to a balanced combination of species and sizes. Prior to testing, fish were seined from holding ponds, held in concrete raceways for at least 48 h, and maintained in good condition on a diet of live or artificial food, depending on the species. During testing, fish were transferred one at a time to the test tank and confined in the area between the two electrodes. After allowing 3-10 s for the fish to orient, and when the fish was positioned perpendicular to the electrodes, the current was switched on for 15 s. As individuals, fish were treated once and to a single power density, but as a group, fish were exposed to power densities incrementing from zero to levels exceeding those needed to immobilize them within 3 s. Power density was incremented by raising voltage at a nearly constant conductivity that varied only due to small changes in temperature that might have occurred during the 1-2 h treatment period. The immobilization response (i.e., within 3 s) was recorded as 0 for no immobilization and 1 if the fish was immobilized. Also, we recorded whether the test fish exhibited no visible response, fright, narcosis, or tetany by the completion of the 15-s period. The 3-s period estimated the time within which if the fish was not immobilized, it would likely escape the electrical field; the 15-s period estimated the maximum amount of time that a fish would be exposed to electricity in an actual field setting. The number of fish tested per treatment ranged from 12 to 41, including 2-3 controls (i.e., no power density applied). All of these fish were used in conjunction with a larger study that evaluated immobilization thresholds; thus, sample size was dependent upon the number of individuals needed to identify immobilization thresholds. The reactions of each fish to the electricity were noted, but were also recorded via a video camera positioned over the tank, allowing review of responses to verify the accuracy of live observations. Following treatment, fish were transferred to separate aerated 38-L holding tanks, and held for 18 h to allow potential hemorrhages to develop, and for determination of short-term mortality.

Injury Assessment

Following the 18-h holding period, the tanks were checked for incidence of fish mortality. Fish that remained alive after the holding period were euthanized in a solution of 100 mg/L MS-222. All specimens were kept on ice for transport to Mississippi State University's School of Veterinary Medicine where they were radiographed within 2 h. Radiographs were examined for evidence of spinal injury (i.e., compression, misalignment, or fracture of the vertebral column). Moreover, a certified radiologist reexamined radiographs to verify interpretation of spinal injury, and to help differentiate among congenital abnormalities, past injuries, and those attributed to the electroshock exposure. Immediately following radiography, all fish were necropsied to evaluate tissue hemorrhage. Necropsy included filleting the length of the body just posterior to the pectoral fins, along the rays and spine, to the caudal peduncle. For reference, digital photographs of all filleted fish (lateral view) were taken. Mortality, spinal injury, and tissue hemorrhage were scored binarily as 0 for no hemorrhage, spinal injury, or mortality and 1 if hemorrhage, spinal injury, or mortality occurred.

Research expenses had not been budgeted for the evaluation of injury in the smallest size class of largemouth bass, bluegill, and channel catfish treated with the 60 Hz treatment. Thus, these fish were not radiographed, but hemorrhage was evaluated when possible. However, we were able to evaluate mortality in all individuals that were not budgeted for injury assessment because there was no cost involved with holding fish overnight.

Data Analyses

Percentage of fish exhibiting hemorrhage, spinal injury, and mortality were calculated according to species-size class combination. These calculations were limited only to fish that were immobilized within 3 s. Fish not immobilized within 3 s were excluded from analyses because electrofishing is commonly conducted with power densities high enough to induce immobilization, and because including fish that were not immobilized artificially reduced overall levels of injury and mortality. Likewise, all subsequent statistical analyses on hemorrhage, spinal injury, and mortality data were conducted only on fish that were immobilized within 3 s.

Effects of fish size, species, and duty cycle.-- The effects of size, species, and duty cycle on percentage hemorrhage, spinal injury, and mortality was evaluated through analysis of covariance (ANCOVA; SAS Institute 1996). The model examined the effect of species, fish weight, duty cycle, and their interactions on the three categories of injury. Fish weight was selected to describe size because it is easy to measure and was shown that it was better correlated with immobilization than length (Dolan and Miranda, in review). To satisfy assumptions of linearity and homogeneity of variances, we transformed the injury response variables (arcsine of square root) and the fish size and duty cycle covariates ($\log_e$, inverse). The ANCOVA model was fit separately to each of the three response variables (i.e., hemorrhage, spinal injury, and mortality). Multiple linear contrasts (SAS Institute 1996) were used to test for differences in hemorrhage, spinal injury, and mortality across significant main effects or interaction variables. We relaxed significance testing to $\alpha = 0.2$ because making a Type II error (i.e., accepting a null hypothesis of no effect when the alternative is true) was a major concern due to the nature of the effect being tested.

Effect of behavioral endpoint on injury and mortality.-- The behaviors displayed by fish at the conclusion of the 15-s period (i.e., narcosis or tetany) were used to categorize injury. Fisher's exact tests (SAS Institute 1996) were applied to test if incidence of hemorrhage, spinal injury, and mortality differed between fish displaying the narcosis and tetany endpoints. Similarly, fish were separated as to whether they lived or died during the 18-h holding period after electroshock treatment, and hemorrhage and spinal injury compared between these two categories with Fisher's exact test.

Results

In all, 884 treatment fish and 93 control fish were tested, ranging in mean total length from 72 to 320 mm (overall mean = 179 mm), and total weight from 4 to 283 g (overall mean = 113 g). Of the treatment fish, 625 (71%) were immobilized within 3 s and included in analyses. Incidence of hemorrhage was evaluated in 81% of the fish immobilized, spinal injury in 77%, and mortality in all of the fish. Water temperatures ranged from 16 to 28° C (mean = 24° C). Specific conductivity was relatively invariable at 195±4 $\mu S/cm^{-1}$ throughout the study. However, due to fluctuations in water temperature, ambient water conductivity (equation 1) ranged from 161 to 213 $\mu S/cm^{-1}$. Peak voltages applied in these waters ranged from 12 to 1,100 V, and peak power densities (equation 2) ranged from 7 to 55,560 $\mu W/cm^3$.

No hemorrhage, spinal injury, or mortality was observed in control fish. In treatment fish, injuries normally occurred mid-dorsally along the vertebral column. Spinal injury usually consisted of the compression of 2-3 vertebrae, without discernible fractures. Hemorrhages ranged from 1-3 vertebrae in diameter. Mortalities occurred over the first 3 h of the 18 h holding period, but is likely that many fish were killed during the 15-s treatment because often fish appeared to not recover from tetanus.

Incidence of hemorrhage averaged 3% and ranged from 0 to 25% (Table 1). The ANCOVA indicated that hemorrhage incidence differed among species ($P = 0.10$), but was not correlated with duty cycle or fish weight. Linear contrasts suggested that largemouth bass had the highest vulnerability to hemorrhage, while bluegill had the least; channel catfish were intermediate between the two species and did not differ significantly from largemouth bass or bluegill.

Incidence of spinal injury averaged 3% and ranged from 0 to 22% (Table 1). No spinal injuries were recorded for small largemouth bass, small bluegill, small channel catfish, or large channel catfish. The greatest levels of spinal injury were exhibited by large largemouth bass and large bluegill. The ANCOVA indicated that spinal injury differed among species ($P < 0.01$), was inversely related to duty cycle ($P < 0.01$) and directly related to weight ($P < 0.01$). Interactions between weight and duty cycle ($P = 0.01$), and species and duty cycle ($P = 0.03$), reflected the absence of injury to small fish and large channel catfish, and the increasing level of injury in large largemouth bass and large bluegill as duty cycle decreased.

Incidence of mortality averaged 10% and ranged from 0 to 75% (Table 1). The ANCOVA indicated that mortality depended on species ($P = 0.02$) and was inversely correlated with weight ($P = 0.01$). However, an interaction between weight and species suggested that the mortality effect of weight depended on species ($P = 0.02$). For largemouth bass and bluegill, mortality increased as weight decreased, but the reverse was true for channel catfish. Moreover, mortality was inversely related to duty cycle ($P < 0.01$), but interaction between duty cycle and weight ($P = 0.02$) indicated that the effect of duty cycle depended on fish weight. This interaction reflected the increased incidence of mortality at low duty cycles for small fish, but a lack of relation across duty cycles for large fish.

Incidence of hemorrhage for fish narcotized by the end of the 15-s period was 1% and 4% for those that were tetanized (difference significant at $P = 0.13$). Similarly, incidence of spinal injury for fish narcotized was 1%, and 3% for fish that were tetanized ($P = 0.47$). Mortality for fish narcotized was 5%, and 14% for fish that were tetanized ($P < 0.01$). Hemorrhages were detected in 4% of fish that survived electrical treatment, but none of the fish that died had hemorrhages ($P = 0.24$). Similarly, incidence of spinal injury was 3% in fish that survived electrical treatment, but none of the fish that died suffered spinal injuries ($P = 0.62$).

Discussion

Although our tank experiments controlled for many sources of error commonly associated with field electrofishing, some estimation error could not be avoided. We strived to maintain the ambient conditions as constant as practicable, but some variability in water temperature had to be accepted owing to the seasonal availability of test fish. Error could have been introduced by the range of experimental temperatures, which possibly influenced fish conductivity and reaction thresholds (Whitney and Pearce 1957), and perhaps incidence of injury and mortality. Moreover, identification of the immobilization response relied on an observer's ability to discern the moment fish were immobilized. As duty cycle decreased, fish exhibited a vigorous forced swimming behavior that sometimes made it difficult to assert whether the fish had been immobilized within 3 s, even after reviewing recorded videos. In addition, in a few instances it was difficult to distinguish among spinal injuries attributable to electroshock, past injuries, and congenital abnormalities. However, errors surrounding spinal injury interpretation were limited due to the validation of questionable injuries by a certified radiologist.

Incidence of electrofishing-induced injury and mortality depended on fish size, species, and duty cycle. Differences due to size are most likely linked to disparities in muscle mass. Moreover, large fish tend to have a higher proportion of white muscle fibers that are larger than red muscle fibers and contract more powerfully (Helfman et al. 1997). Larger fish often have more developed muscles that contract forcefully and may severely compress the vertebrae to cause spinal injury and associated hemorrhage (Lamarque 1990). We observed spinal injury and hemorrhage most often on or near the vertebral column in dorsal-anterior regions of the trunk, where the strongest muscle contractions were likely to occur. Differences due to species probably derive from anatomical and morphological attributes linked to species adaptations. For example, channel catfish possess a dense, heavy skeleton (Evans 1998) to support a benthivorous feeding strategy (Pflieger 1997), whereas fish that feed within the water column such as bluegill and largemouth bass have a lighter, poorly ossified skeleton. Differences due to duty cycle reportedly reflect changes in response to varied electrical stimuli by the fish's nervous system (Lamarque 1990; Sharber et al. 1994; Sharber and Black 1999). Nevertheless, in practical field electrofishing differences among species are difficult to control because electrofishing affects all species that are exposed to the electrical field, but the user can have complete control over duty cycle and some control over the size of fish targeted.

Duty cycle can be controlled by manipulating pulse frequency and pulse period using the instrumentation provided by most commercial electrofishing equipment. As duty cycle is decreased, increasingly higher power densities are required to immobilize fish (Miranda and Dolan, in review), which in itself may be sufficient to cause injury. But in addition, as duty cycle decreases there is a decreasing margin of difference between the electrical power required to narcotize fish and that required to tetanize them, to the extent that at 0.015 duty cycle fish cannot be immobilized unless they are also tetanized

(Miranda and Dolan, in review). In addition, we observed that fish exposed to low duty cycles vibrated or quivered vigorously. This vibration was consistent with the symptoms (twitches, jerks, and convulsions) of epileptic seizure described by Sharber and Black (1999), who stated that seizures could be induced in many vertebrates (including fish) by passage of electrical current through the brain. Epileptic seizure has been suggested as cause for gross physical injuries such as spinal injury (Sharber et al. 1994), and it follows that seizures also may result in less detectable injuries (e.g., organ, tissue, and cell injury) that may eventually lead to death. High power density requirements, seizures, coupled with the need to tetanize fish to achieve immobilization (more about detriments of tetany below), likely contribute to the harmful effects of low duty cycles. Because spinal injury and mortality were inversely related to duty cycle, electrofishing with high duty cycles should minimize detrimental effects.

Substantially higher power densities are required to immobilize small than large fish (Dolan and Miranda, in review). These discrepant requirements normally translate into unequal size efficiencies, with electrofishing being more effective for immobilizing large fish (Bayley and Austen 2002; Dolan and Miranda, in review). Limiting power output to that necessary for immobilizing large fish only, could potentially reduce or eliminate high levels of mortality associated with electrofishing small fish with low duty cycles. Nevertheless, the electrical field created between electrodes under field conditions is heterogeneous, varying in intensity by several folds, depending on electrode sizing and positioning (Reynolds 1996). Consequently, while the operator can control the power transmitted between electrodes, often may not be able to control exposing fish to high power densities that are commonly encountered in the proximity of electrodes. Novotny (1990) identifies approaches for reducing excessive field intensities surrounding electrodes.

Traditionally, field electrofishing has been considered most effective when conducted with settings that have maximum tetanizing effects (Lamarque 1990). Our observations indicate that regardless of duty cycle, tetanized fish exhibited more injury and mortality. Persistence of tetany after the interruption of current may prevent resumption of respiration, leading to suffocation and death. Given that injury levels were less in fish that were narcotized but not tetanized, operating equipment to produce power densities that induce narcosis rather than tetany can reduce injury and mortality. This alternative operational approach simplifies electrofishing by requiring adjustment of electrical output through observation of fish behavior in the electrical field, rather than through in-water measurement of electrical variables.

Hemorrhage and spinal injury were higher in fish that initially survived electroshock versus those that died. This finding implies that the mechanisms that cause the gross physical injuries, such as hemorrhage and spinal injury, may not be the same as those that cause immediate mortality. Similarly, Taylor et al. (1957) found that electrofishing mortality of rainbow trout was almost never associated with ruptured blood vessels, injury to bones and organs, or other trauma. Spencer (1967) reported a lack of correlation between incidence of spinal injury and mortality of bluegills, and Hudy (1985) stated that more vertebral injuries were observed in trout that survived electroshock versus those that died. Many of the mortalities in our experiments appear to have occurred rapidly within the 15-s treatment period. Potentially, rapid immobilization and death prevented the physical stress experienced by fish that survived.

Minimizing the risk of harm to fish during population surveys is clearly an important goal of fish sampling. Our results suggest that electrofishing with continuous DC, or with pulsed DC with high duty cycles, may be the best choices for reducing electrofishing harm to warmwater fish. Power output should be managed to induce narcosis and avoid tetany, because tetany is associated with higher injury rates. Tetany is avoided easier with high duty cycles because of a wider margin of difference between the electrical power required to narcotize fish and that required to tetanize them. Manipulation of maximum power output to target immobilization of large fish while excluding small fish would reduce overall mortality rates. However, modifications to the electrode system may be necessary to avoid high power densities that commonly occur in the proximity of electrodes.

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Table 1. Incidence (%) of hemorrhage, spinal injury, and mortality for 625 fish treated with various duty cycles and with power densities high enough to immobilize them within 3 s. Values within [ ] represent the frequency-pulse period combination, within { } the mean total length (mm) and weight (g), and within ( ) the number of fish treated with each duty cycle. Blank = not tested. No hemorrhages, spinal injury, or mortalities were observed in 93 control fish.

Species and injury	Duty cycle							
	1 [DC]	0.660 [110-6]	0.360 [60-6]	0.120 [CPS™]	0.110 [110-1]	0.090 [15-6]	0.060 [60-1]	0.015 [15-1]
Channel catfish {162, 30}	(17)	(14)	(18)	(10)	(16)	(17)	(15)	(11)
Hemorrhage	6	0		0	0	0		0
Spinal injury	0	0		0	0	0		0
Mortality	0	0	0	10	0	6	0	0
Channel catfish {320, 283}	(16)	(14)		(14)	(15)	(15)		(13)
Hemorrhage	6	0		0	20	0		0
Spinal injury	0	0		0	0	0		0
Mortality	6	14		29	0	0		15
Bluegill {67, 5}	(10)	(13)	(34)	(10)	(14)	(23)	(15)	(14)
Hemorrhage	0	0		0	0	0		0
Spinal injury	0	0		0	0	0		0
Mortality	0	0	0	50	0	52	7	50
Bluegill {156, 80}	(12)	(13)		(10)	(12)	(18)		(12)
Hemorrhage	0	0		0	8	0		0
Spinal injury	0	0		20	0	0		8
Mortality	0	0		0	8	0		0
Largemouth bass {72, 4}	(11)	(12)	(14)	(12)	(17)	(24)	(14)	(13)
Hemorrhage	0	17	14	0	25	0	7	0
Spinal injury	0	0		0	0	0		0
Mortality	0	0	29	75	0	54	14	46
Largemouth bass {216, 135}	(13)	(9)		(10)	(13)	(9)		(11)
Hemorrhage	8	0		10	0	0		0
Spinal injury	0	0		10	15	22		18
Mortality	0	0		0	0	0		18
Largemouth bass {262, 253}					(12)		(16)	
Hemorrhage					0		6	
Spinal injury					8		0	
Mortality					0		0	

Efficiency of Electrofishing Relative to Fish Size

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Abstract.— Under controlled laboratory conditions, and using several waveforms, we measured the electrical power required to immobilize an array of fish species of diverse sizes and shapes. Size was indexed by total body length, area, volume, and weight, and shape by the ratio of body length to body depth. Our objectives were to identify immobilization thresholds, elucidate what descriptors of fish size were best associated with efficiency of electrofishing, and determine if the vulnerability of a species relative to other species remained unchanged as waveform changed. Our results confirmed that fish size is a key variable driving immobilization efficiency by electrofishing, and suggested that the size descriptor best related to electric power was fish volume. Power needed to immobilize fish decreased rapidly with fish volume in small fish, but decreased slowly for fish larger than 75-100 cm^3 (15 cm). This differential size effectiveness demands careful interpretation of survey data when electrofishing is used to make inferences about population structure because larger and older individuals are selected, or community structure because larger species are selected. Our study showed that different settings did not favor different species, possibly because of the overwhelming effect of body size.

The efficiency with which electrofishing immobilizes fish has often been linked to fish size (Zalewski and Cowx 1990; Reynolds 1996). Most such studies have shown that large fish are easier to catch than small ones (e.g., Reynolds and Simpson 1978; Zalewski 1985; Buettiker 1992; Anderson 1995). The effect of size is generally linked to body length (e.g., Taylor et al. 1957; Adams et al. 1972), but some authors have acknowledged the effect of total surface area (Emery 1984) and body form (Zalewski 1983). A few explanations have been offered to explain observed differences in electrofishing efficiency relative to size. Most authors (e.g., Vibert 1967; Reynolds 1996) concur that the vulnerability of a particular fish species to electroshock increases with fish length because at a given voltage gradient, total body voltage increases as length increases (i.e., head-to-tail voltage is greater for large fish). Lamarque (1967) further explained that large fish have long nerves that require low voltage to stimulate (Rushton 1927). Nevertheless, Lamarque and Charlon (1973) showed that the voltage threshold for stimulation remains stable for fish nerves longer than 4 cm, suggesting that the effect of nerve length might be important for small fish, but would become trivial as fish size increases. Halsband (1967) considered size effects relative to pulsating currents, and suggested that large fish are immobilized more efficiently because they have large muscles, the size of which prevents slackening between high-frequency pulses.

Disparities in immobilization efficiency over various electrical settings have been reported for some species. Halsband (1967) claimed that electricity pulsed at 90 Hz was most effective for immobilizing small cyprinids, 80 Hz for salmonids, 50 Hz for common carp *Cyprinus carpio*, and 20 Hz for eels *Anguilla anguilla*. Novotny and Priegel (1974) suggested that 15-40 Hz was effective for fish such as walleye *Stizostedium vitreum*, yellow perch *Perca flavescens*, white bass *Morone chrysops*, and bluegill *Lepomis macrochirus*; 40-120 Hz was effective for fish such as salmonids, largemouth bass, and common carp; and 80 to 120 Hz effective for bullheads *Ameiurus* spp. In contrast, Corcoran (1979) reported that most ictalurids were best immobilized by 20 Hz, and Gilliland (1987) confirmed that 20 Hz was most effective for flathead catfish *Pylodictis olivaris*. The physiological basis for catfish response to low-frequency pulses is not fully understood, but is reportedly related to the ability of the fish to sense weak electrical fields displayed by some ictalurids (Peters and Bretschneider 1972). Emery (1984) stated that species with large scales were better insulated from electricity than those with small or no scales, and that increasing pulse frequency favored immobilization of small fish. Miranda and Schramm (2000) reported significant differences in species assemblages measured with two frequencies of pulsed DC in the Mississippi River.

Under controlled laboratory conditions, we measured the electrical power needed to immobilize an array of fish species of diverse sizes and forms. These immobilization thresholds were then analyzed relative to species, an index of fish form, and several descriptors of fish size. The objectives of these analyses were to identify immobilization thresholds for the selected fish species, to document the relation between fish size and immobilization threshold, to elucidate what descriptors of fish size are best associated with efficiency of electrofishing, and to determine if the vulnerability of a species relative to other species remains unchanged as test electrical settings change.

Methods

Test Equipment

All testing was conducted indoors in a polyethylene tank 2.0 m long, 1.5 m wide, and 1 .0 m deep. The tank was filled to a depth of 10 cm with well water. The cross sectional profile of the tank was equipped with two, 1.6-cm thick aluminum plate electrodes positioned 65 cm apart, perpendicular to the longitudinal axis of the tank. Electricity was supplied to the plates via a Smith-Root 15-D POW unit (Smith-Root, Inc., Washington) modified to allow continuous rather than discrete voltage control, and equipped with additional smoothing capacitors to eliminate spikes and reduce ripples at the peak of the pulses (i.e., ripples averaged ± 6% of the amplitude). Conditions within the tank produced a homogeneous electrical field with a constant voltage gradient. Specific conductivity (C_s, $\mu S \cdot cm^{-1}$) and ambient water temperature (T_w) were recorded with a YSI 30/10 FT meter (Yellow Springs Instruments, Ohio). The meter read C_s at specific temperature (T_s, 25° C). Ambient water conductivity (C_w) was estimated from specific conductivity, specific temperature, and ambient water temperature (Reynolds 1996) as

$$C_w = C_s \cdot 1.02^{T_s - T_w} \tag{1}$$

Electrical Treatments

Five electrical treatments were considered, including no pulse direct current (DC), and pulsed DC with 110 or 15 Hz and 1 or 6 ms pulse periods. These pulse frequencies and periods were selected because they represented settings near the upper and lower range of settings commonly available in commercially available units. Peak voltage (V_{pk}), root-mean-square voltage (V_{RMS}), frequency, and pulse period were measured within the energized field with a Tektronix THS720A oscilloscope (Tektronix, Inc., Oregon). Peak voltage measures maximum amplitude attained by a pulse, while RMS voltage quantifies the equivalent uninterrupted DC voltage applied by different forms of pulsed DC (Kane and Sternheim 1988). Because the pulses were rectangular, V_{RMS} was a fixed fraction of V_{pk}. For DC, pulsed DC with 110 Hz and 6 ms (notated as 110-6), pulsed DC 110-1, 15-6, and 15-1, V_{RMS} values were 1, 0.762, 0.350, 0.272, and 0.128 of V_{pk} values, respectively. Following Kolz and Reynolds (1989), V_{pk} was used to calculate power density (P_w) as

$$P_w = C_w \cdot \left(\frac{V_{pk}}{d} \right)^2 \qquad (2)$$

where d is the distance between the electrodes (i.e., 65 cm). Nevertheless, the equivalent power density based on V_{RMS} measurements, herein termed RMS power density, can be derived as the product of P_w and the V_{RMS} fraction squared (e.g., for 110 Hz, 6 ms, RMS power density = 0.762^2 x P_w).

Test Fish

We applied the five electrical treatments to 12 species-size combinations selected because they were readily available from fish culture facilities or local streams (Table 1). Nevertheless, limited fish availability permitted application of all five treatments to only eight of the 12 species-size combinations; thus, some species were not treated to all settings. Prior to testing fish were seined from culture ponds or from streams, held in concrete raceways or polyethylene circular tanks for at least two weeks, and maintained in good condition on a diet of live or artificial food, depending on the species. During testing, fish were transferred one at a time to the test tank and confined in the area between the two electrodes. After allowing 3-10 s for the fish to orient, the current was switched on when the fish positioned perpendicular to the electrodes. As individuals, fish were treated only once and to a single voltage, but as a group, fish were exposed to voltages incrementing from near zero to levels exceeding those needed to immobilize them within 3 s. The immobilization response was recorded as 0 for no immobilization and 1 if the fish was immobilized. As many as 18-35 fish were used per treatment, depending on ease of identifying immobilization threshold. The reactions of each fish were observed and written down, but were also recorded via a video camera positioned over the tank, allowing review of responses to verify the accuracy of live observations.

Immobilization Thresholds

Field strength has traditionally been measured as voltage gradient, current density, or power density (voltage gradient X current density). More recently, Kolz (1989) suggested that the success of electrofishing depends on the fraction of the power density that is transferred to the fish. The power transfer model has been shown to reduce variability of survey data (Burkhardt and Gutreuter 1995) and to adequately predict power levels required to elicit immobilization of fish over a wide range of water conductivities (Kolz and Reynolds 1989; Miranda and Dolan, in review).

For each electrical treatment the dependent binary immobilization response y_i recorded for each fish was regressed on the independent variable P_w applied to each fish with the logistic regression model

$$y_i = \beta_o + \beta_i F + \beta_1 \log_e P_w \qquad (3)$$

where β_o represents the intercept parameter, $\beta_i F$ the differential effect attributed to the species-size category, and β_1 the slope parameter for $\log_e P_w$. The resulting logistic model was used to predict the peak power density threshold required for a 0.95 probability of immobilization ($P_{w,\,0.95}$) as

$$P_{w,\,0.95} = e^{y_i}\,(1 + e^{y_i})^{-1} \qquad (4)$$

The predicted $P_{w,\,0.95}$ was then used to estimate the power transferred into the fish ($P_{f,\,0.95}$) as

$$P_{f,0.95} = P_{w,0.95}\left(4\frac{C_f}{C_w}\right)\left(1+\frac{C_f}{C_w}\right)^{-2} \qquad (5)$$

where C_f is the estimated standard conductivity of fish (115 S cm^{-1}) suggested by Miranda and Dolan (in review).

Effect of Body Size and Form

Three direct and one indirect measure of body size were considered. Direct measures included total length (tip of nose or mandible to tip of caudal fin), total area (including all fins), and total volume (including all fins). Total length was measured for all fish after they were euthanized the day following treatment. Total area and volume were measured for a sample of fish representative of the average size included in each treatment. Area and volume were estimated from digital photographs of a subsample of fish, using Image Tool software (University of Texas Health Science Center, San Antonio). Total wet weight was considered an indirect measure of body size because it manifests total body size but not its proportions. Additionally, body depth was measured as the maximum vertical distance in the fish's body (excluding fins), and used to construct an index of body form as total length/total depth.

The relation between $P_{f,\ 0.95}$and the fish size variables was examined through analysis of covariance with the model

$$\log_e P_{f,\ 0.95} = \beta_o + \beta_1 \log_e S_i + \beta_2 H_i + \beta_j E \qquad (6)$$

where β_o represented the model's intercept parameter, β_1 the slope parameter for size of the i^{th} species, β_2 the slope parameter for form index H of the i^{th} species, and $\beta_j E$ the effect of the j^{th} electrical setting. Interactions between the three main effects were also included. Equation 6 was fit separately to the S_i descriptors total length, total area, total volume, and total weight. Once aptness of each model was verified by residual analyses, the degree of association between $P_{f,\ 0.95}$ and the S_i variables was indicated by the coefficient of determination (R^2).

Effect of Species

We tested if the vulnerability of a species relative to other study species remained constant over electrical settings. This analysis was limited to the four species for which all five treatments were applied (i.e., all sizes of black crappie, bluegill, channel catfish, and largemouth bass). The effect of species was tested with the analysis of covariance model

$$\log_e P_{f,\,0.95} = \beta_o + \beta_i F + \beta_j E + \beta_1 \log_e S_i \qquad (7)$$

where β_o represented the model's intercept parameter, $\beta_i F$ the effect of the i^{th} species, $\beta_j E$ the effect of the j^{th} electrical setting, and β_1 the slope parameter for the fish size variable S_i. Equation 7 was fit with the S_i descriptor identified to maximize R^2 in equation 6. Of interest was the interaction between the class variables, F and E. A significant interaction would signal that vulnerability of a species relative to other species changed with electrical settings, whereas a non-significant interaction indicates vulnerability of the species relative to each other remained relatively unchanged among electrical settings.

Results

The 1,240 fish included in these tests encompassed a wide range of sizes encountered in fresh water. Their mean total length ranged 5-33 cm, area 4-169 cm^2, volume 2-336 cm^3, weight 1-285 g, and length/depth ratio 2.8-7.7 (Table 1). Larger fish were not included because of physical limitations imposed by the experimental conditions. Species with smaller or larger length/depth ratios were not available.

Although we strived to maintain ambient conditions as constant as practicable to focus on the effect of fish size, some variability in water temperature had to be accepted owing to the seasonal availability of test fish. Water temperatures at which fish were held and tested ranged 17-27°C and averaged 22.4°C. Some error could have been introduced by this range of experimental temperatures that possibly influenced fish conductivity and fish reaction thresholds (Whitney and Pierce 1957). Whereas specific conductivity was a relatively invariable 194 $S \quad cm^{-1}$ throughout the study, due to fluctuations in water temperature specific water conductivity (equation 1) ranged 176-201 S cm^{-1}. Peak voltages applied in these water conditions ranged 12-1,100 V, and peak power densities ranged 7-147,500 W cm^{-3}.

Estimates of the amount of power that needs to be transferred to immobilize 95% of the fish treated ranged from as high as 88,635 W cm^{-3} for immobilization of darters with pulsed DC 15-1, to as low as 28 W cm^{-3} for large-bodied fish of several species treated with pulsed DC 110 Hz and DC. While levels of P_f, $_{0.95}$ (W cm^{-3}) decreased as fish size increased, total power transferred to fish (W) increased with fish size (Figure 1). Decreases in $P_{f,\ 0.95}$ relative to size were large for small fish, but small for large fish. The effect of size on immobilization became minor when fish volume reached 75-100 cm^3, or roughly 15 cm. $P_{f,\ 0.95}$ was not affected (P = 0.93) by the index of body form within the range of forms included in this study, but was highly influenced by all the body size variables considered. After the body form parameter and variable (i.e., β_2 H_i) were removed from equation 6, length accounted for 0.909 of the variability in $P_{f,\ 0.95}$, area for 0.927, weight for 0.929, and volume for 0.951 (Table 2). Vulnerability of largemouth bass, bluegill, black crappie, and channel catfish in relation to each other remained relatively constant over electrical settings as seen in Figure 1 and indicated by a lack of significant (P = 0.55) interaction between the class variables *F* and *E* in equation 7.

Peak power necessary to immobilize fish differed among electrical treatments. Power requirements were highest for pulsed DC 15-1, followed by pulsed DC 15-6, DC, pulsed DC 110-6, and least for pulsed DC 110-1 (Table 2). In terms of RMS, power requirements were highest for DC followed by pulsed DC 15-1, 110-6, 15-6, and least for PDC 110-1.

Discussion

Immobilization efficiency by electrofishing was inversely related to size, particularly body volume. Under power transfer theory (Kolz 1989), body volume is intuitively a relevant variable because power density applied into the water and power transferred into the fish are both expressed in units of volume. Nevertheless, body length, area, and weight were also correlated with immobilization efficiency, which is expected given that these measures of body size are tightly correlated with body volume.

The lack of relationship between immobilization efficiency and body form reinforces the notion that volume may be the principal body size variable controlling electrofishing efficiency. The body forms considered in this study comprised most of the forms found in fresh water. Fish ranged from laterally compressed forms such as *Lepomis* and *Pomoxis*, to elongated forms such as *Micropterus* and *Etheostoma*. Flatfishes do not occur in fresh waters of North America, but we did include adult *Ictalurus* that have an elongated body with a depressed head. Missing from our study were the highly elongated fish such as *Esox* that have length-depth ratios near 7-8, and *Lepisosteus* that have ratios near 11-14 and higher for juveniles. Given the total lack of relation between immobilization efficiency and the 2.8-7.7 range in the index, we hypothesize that inclusion of more elongated fish would not change our conclusions, or at best would only identify an unpromisingly weak relation.

The DC treatment caused fish to exhibit forced swimming towards the anode (i.e., positive electrode) before being immobilized within 3 s. This attraction occurred immediately upon electrification of the field, was conspicuous in some species but occurred in all species and sizes treated with DC. However, at power levels well above those needed to immobilize fish within 3 s, immobilization occurred instantly once the field was electrified, with no obvious forced swimming towards the anode. Attraction towards the anode was observed in a few fish treated with pulsed DC, but was not as striking as with DC.

Power densities needed to immobilize fish decreased rapidly with fish size in small fish, but decreased slowly for fish larger than 75-100 cm^3 (15 cm). Taylor et al. (1957) investigated the response of 3-34 cm rainbow trout to DC electrofishing in a homogeneous field, and reported decreasing response thresholds as length increased to 25 cm, but no clear threshold difference among longer fish. Similarly, Anderson (1995) found that probability of capturing brown trout *Salmo trutta* increased until fish reached 20-25 cm, and afterward increments were negligible. Zalewski (1985) collected multiple species and showed that the electrofishing capture probability in streams increased rapidly with fish size for small fish, but increases became minor when fish reached about 50 g, consistent with the deceleration recorded in our trials. Our findings suggest that electrofishing cannot adequately sample the entire length or age structure of a species with an extended size range. However, it can provide a less biased representation of the large sizes of large-bodied species, and possibly species with concise size distributions that exhibit small differences in size between juveniles and adults (e.g., *Etheostoma*). Likewise, because of its size-selectivity, electrofishing is unlikely to accurately portray community composition of fish assemblages with a mixture of small and large species.

The vulnerability of a species relative to other species remained constant over the five test electrical settings. Thus, when accounting for fish size, there was no indication that a setting favored one species while another setting favored a different one. These results contradict accounts about species selectivity summarized early in this article. There are several plausible explanations for this apparent discrepancy. Conceivably, our experiment did not include enough species or enough electrical settings to detect species differences. We believe this argument is shaky because a wide range of electrical settings were included, and the species considered varied greatly in size and biological characteristics and included catfish, reported to have atypical reactions to electricity (Corcoran 1979; Gilliland 1987). Alternatively, our homogeneous electric field may not adequately recreate typical conditions. In a heterogeneous field, fish are exposed to power density gradients that expand below and above the immobilization threshold, which could stimulate species differently and produce the differences reported by other authors.

It is also plausible that many of the differences previously attributed to species may simply represent the effect of disparities in body size. Increased efficiency towards small fish attributed to high pulse frequencies (Emery 1984) may just reflect the higher mean power afforded by high-frequency pulses. We were unable to test Emery's (1984) allegation that fish with large scales are better insulated from electricity, but the little evidence we have is not corroborative. Kolz and Reynolds (1989) reported that when electrofishing with DC, 179 W cm^{-3} were needed to immobilize goldfish *Carassius auratus*, a species with relatively large cycloid scales, averaging 7.5 cm and 5.3 g. Although their estimation methods were different from ours, their estimate fits roughly near the levels observed for 6.7 cm bluegill in the top panel of Figure 1, and provides no indication that goldfish would be less susceptible to electrofishing. Because the power required to immobilize fish depends on pulse frequency, strength of the effective electric field created during electrofishing, and hence the radius of action, varies with pulse setting if the power source is operating near its limits, potentially producing differences in species catch rate, such as those noted by Miranda and Schramm (2000). Additionally, we found that low-frequency pulses with short period required high power levels to immobilize fish, thus tended to encourage forced swimming and thrashing rather than immobilization in all species tested. This observation is consistent with those made by Gilliland (1987) who reported that such pulses made the fish easier to detect, but that collection often required a chase boat because fish were not immobilized. Corcoran (1979) found that longer pulse periods caused fish to remain at the surface longer, consistent with the observation that such pulse setting carries more mean power. Although some species differences exist due to differences in resistivity (Miranda and Dolan, in review), swimming ability (Novotny and Priegel 1974), and other species peculiarities (e.g., Holliman 1998), most of the variability in immobilization efficiency can be accounted for by fish size.

In conclusion, our study confirmed that fish size is a key variable driving immobilization efficiency by electrofishing, and suggested that the size descriptor best related to electric power was fish volume. Further, whereas the amount of power (μW cm^{-3}) needed to immobilize fish decreased with volume, the total power (μW) transferred into the fish increased, perhaps accounting for the easier immobilization of larger fish. Body form was not a factor. This differential size effectiveness demands careful interpretation of survey data when electrofishing is used to make inferences about population structure because larger and older individuals are selected, or community structure because larger species are selected. Our study further suggests that different settings did not favor species, possibly because of the overwhelming effect of body size. There is much that we don't understand about electrofishing, particularly physiological responses. Without a better understanding of fish physiology relative to electrified fields, results from experiments are difficult to interpret, and pertinent hypotheses to accelerate the rate of knowledge acquisition are difficult to postulate. The science of electrofishing resides in the fringes of fish physiology, electrical sciences, and fishery science; rapid acquisition of knowledge requires successful collaboration among these disciplines.

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Table 1. Fish species-size combinations selected for study. Fish were obtained from aquaculture facilities, a local stream, and a private fish farm. Values represent means for fish of various size groups.

Size group	Length (cm)	Area (cm^2)	Volume (cm^3)	Weight (g)	Length/ depth ratio	Source
			Black crappie (*Pomoxis nigromaculatus*)			
One group	15.3	79.6	80.0	49.2	3.4	MSUAC[1]
			Bluegill (*Lepomis macrochirus*)			
Small	6.8	17.4	12.5	5.0	3.4	MSFH[2]
Large	15.8	92.9	105.7	82.4	2.8	Private producer[3]
			Bluntnose minnow (*Pimephales notatus*)			
One group	5.8	5.3	2.3	1.8	5.6	Catalpa Creek[4]
			Channel catfish (*Ictalurus punctatus*)			
Small	6.2	7.4	4.5	1.8	7.2	MSUAC
Medium	16.3	29.9	30.8	30.0	7.0	MSUAC
Large	31.9	163.9	318.2	280.8	6.6	MSUAC
			Creek chub (*Semotilus atromaculatus*)			
One group	6.2	7.4	4.1	2.6	5.8	Catalpa Creek
			Hybrid striped bass (*Morone chrysops x M. saxatilis*)			
One group	17.6	73.4	98.4	71.0	4.5	MSUAC
			Largemouth bass (*Micropterus salmoides*)			
Small	7.4	11.8	5.5	4.6	7.4	TNFH[5]
Large	21.7	124.8	185.8	138.6	4.6	TNFH
			Redfin darter (*Etheostoma whipplei*)			
One group	5.3	5.1	3.1	1.4	7.7	Catalpa Creek

[1] Mississippi State University Aquaculture Center
[2] Meridian State Fish Hatchery, Mississippi Department of Wildlife, Fisheries and Parks
[3] Calhoun County, Mississippi
[4] Oktibbeha County, Mississippi
[5] Tupelo National Fish Hatchery, U.S. Fish and Wildlife Service

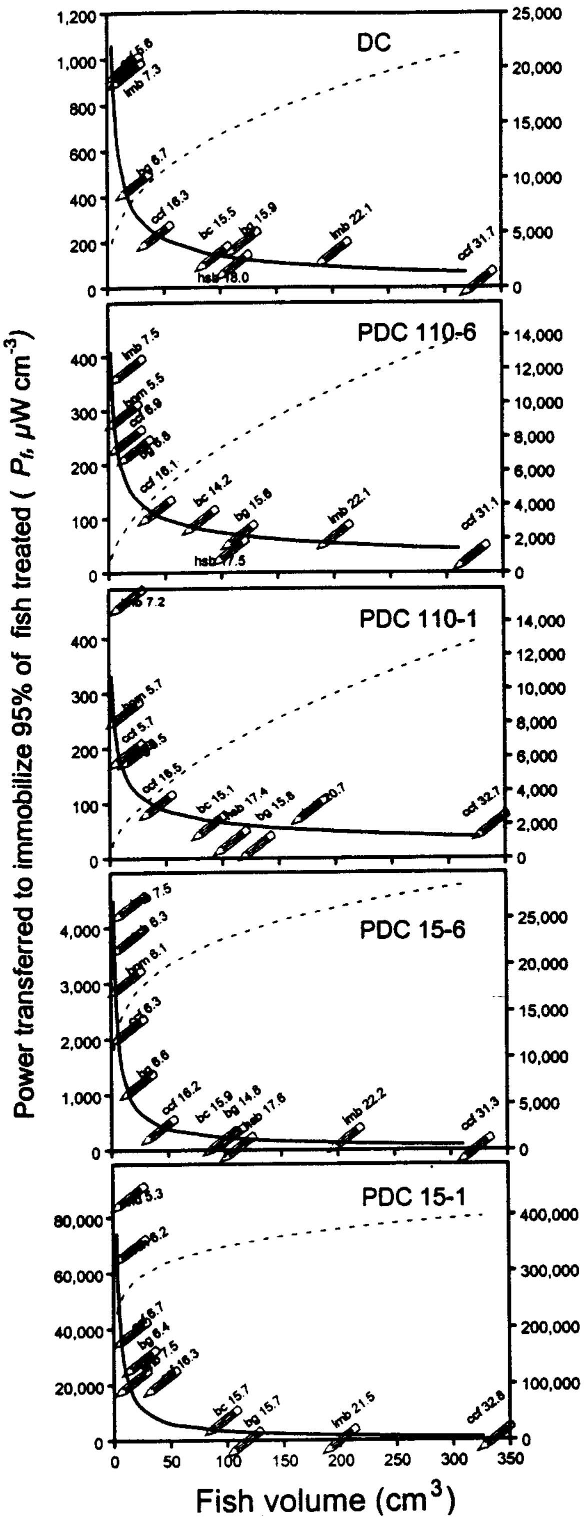
Table 2. Analysis of covariance models descriptive of $P_{f,\,0.95}$ (W cm^{-3}) relative to fish size S_i and the j^{th} electrical setting $\beta_j\,E$. The four body size models are of the form $\log_e P_{f,\,0.95} = \beta_o + \beta_1 \log_e S_i + \beta_j\,E$. For example, $\log_e P_{f,\,0.95}$ for a 100 cm^3 fish treated with DC is $\log_e P_{f,\,0.95} = 8.601 - 0.643 \log_e 100 - 0.718 = 4.922$, and $P_{f,\,0.95} = e^{4.922} = 137$ W cm^{-3}. Values of $\beta_j\,E$ within the same column that do not have a letter in common are significantly different (P < 0.01).

Model parameter		Body size descriptor S_i			
		Length (cm)	Area (cm^2)	Volume (cm^3)	Weight (g)
β_o		10.790[a]	9.494[a]	8.601[a]	8.185[a]
$\beta_1 \log_e S_i$		-1.746[a]	-0.879[a]	-0.643[a]	-0.582[a]
$\beta_j\,E$					
	DC	-0.789v	-0.722v	-0.718v	-0.774v
	PDC,110,6	-1.584w	-1.557w	-1.566w	-1.597w
	PDC,110,1	-1.733x	-1.702x	-1.706x	-1.715x
	PDC,15,6	0.000y	0.000y	0.000y	0.000y
	PDC,15,1	2.822z	2.826z	2.842z	2.817z
R^2		0.909	0.927	0.951	0.924

[a] P <0.001

Figure Caption

Figure 1. The solid curves and circles identify the relation between power transferred to immobilize 95% of fish treated (y-axis 1) and fish volume (x-axis) for five electrical treatments. The dashed curves identify the total power transferred to fish (y-axis 2), calculated as the product of y-axis 1 and x-axis. Labels next to each point represent fish mean length (cm) and species name (bc = black crappie, bg = bluegill, bnm = bluntnose minnow, ccf = channel catfish, cch = creek chub, hsb = hybrid striped bass, lmb=largemouth bass, and rfd = redfin darter).



DC
PDC 110-6
PDC 110-1
PDC 15-6
PDC 15-1
Power transferred to immobilize 95% of fish treated (P_t, μW cm^{-3})
Total power transferred to immobilize 95% of fish treated (μW)
Fish volume (cm^3)

Figure 1.

Test of a Power Transfer Model for Standardized Electrofishing

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Abstract.-- Standardization of electrofishing in waters with differing conductivities is critical when monitoring temporal and spatial differences in fish assemblages. A model that can help improve consistency of electrofishing by allowing control over the amount of power that is transferred to the fish was tested. The primary objective was to test whether the model adequately described fish immobilization responses elicited experimentally with various electrical settings over a range of water conductivities. The model adequately described empirical observations over conductivities ranging from 12 to 1,030 $\mu S \cdot cm^{-1}$, and for various DC and pulsed DC settings ($R^2 \geq 0.92$). The model required knowledge of fish conductivity, an attribute that is likely to vary according to species, size, season, and other variables. Thus, a second objective was to gather available estimates of fish conductivity to examine the magnitude of their variation, and to assess whether in practical applications an average fish conductivity value may be assumed. An average fish conductivity of 115 $\mu S \cdot cm^{-1}$ introduced relatively little error in the estimation of power.

Electrofishing creates a series circuit wherein electricity is transferred from a power source through a metal electrode to the water into a fish and back through the water into another electrode. The magnitude of the power applied to the water (Monan and Engstrom 1963; Adams et al. 1973), and the fraction that is transferred from the water to the fish (Kolz 1989), reportedly dictates the success of electrofishing. Too little power precludes immobilization of the fish, whereas too much power may immobilize the fish before it can be detected by the collector, or may injure the fish (Reynolds 1996). The effectiveness with which power is transferred depends on many variables, including conductivity of the water and conductivity of the fish (Reynolds 1996).

The electrical power carried through a series circuit is optimized when the electrical resistances of loads in the circuit are equal (Joule's Law; Kolz 1989). Accordingly, in electrofishing the power transferred into a fish is optimized when the resistivity of the water and the fish match. When the water has more resistance than fish (i.e., the water is less conductive than fish), current tends to flow through the fish; when the fish has more resistance than water (i.e., the fish is less conductive than water), current tends to flow through the water. In either of these mismatches, the power transferred through the circuit into the fish is less than optimal.

Kolz (1989) proposed a model that adjusted the power applied to the water by compensating for the inefficiency of transfer to the fish. The model relies on differences in the conductivities (ability to carry an electrical current) of fish and water to estimate power to be applied to water with differing conductivities to deliver a constant electric power to fish. Kolz's model is being adopted to standardize electrofishing in management and research applications (e.g., Burkhardt and Gutreuter 1995; Chick et al. 1999). Nevertheless, the model has remained untested except for the work of Kolz and Reynolds (1989). Accordingly, the first objective of this study was to test whether Kolz's model adequately described fish immobilization responses elicited experimentally with various electrical settings over a range of water conductivities.

Standardization of electrofishing in waters with differing conductivities is critical when this gear is used to monitor temporal and spatial changes in fish assemblages. If Kolz's power model is adequate, it may facilitate standardized electrofishing by allowing control over the amount of power that is transferred to the fish. Fundamental to Kolz's model is the knowledge of fish and water conductivity. Conductivity quantifies the ability of a substance to carry an electrical current. Conductivity of water is affected by ionic concentration and composition, and by temperature, and can be readily measured with an electronic meter. Conductivity of fish is affected by similar variables, but direct measurement is complicated by the variety of electrically dissimilar tissues and fluids, along with a nervous system that functions akin to a capacitor. Therefore, Kolz (1989) proposed circumventing measurement of the internal makeup and reaction mechanisms of fish that dictate their conductivity, and instead measure effective conductivity, which is a measure of the behavioral response of a fish to an electrical stimulus.

Fish conductivity is likely to vary according to species, size, season, and other variables. Because sampling with electrofishing generally targets several species and sizes at once, use of species-specific fish conductivity values is not practical. However, a generalized value that represents several fish species may be useful, although the merit of an average value would depend on its precision (i.e., the observed range of fish conductivities). Accordingly, the second objective of this study was to gather available estimates of fish conductivity to examine the magnitude of their variation, and to assess whether in practical applications an average fish conductivity value may be assumed to partially standardize electrofishing.

Methods

Kolz' Model

Kolz (1989) postulated the following model based on electrical theory

$$\frac{P_w}{P_f} = \frac{\left(1 + \frac{C_f}{C_w}\right)^2}{4\frac{C_f}{C_w}} \tag{1}$$

where

P_w = power density applied to the water

P_f = portion of P_w that actually is transferred into the fish,

C_w = conductivity of water, and

C_f = conductivity of fish.

Power density is the product of water conductivity and voltage gradient

$$P_w = C_w \cdot \left(\frac{V}{d}\right)^2 \tag{2}$$

where d is the distance between electrodes and V is voltage. According to equation 1, if $P_w = P_f$, then $C_w = C_f$. P_w may be applied in various forms, including direct current (DC) and pulsed DC.

Electrical Treatments

Six electrical treatments consisting of a range of pulse frequencies, and 14-18 water conductivity levels were considered. The electrical treatments were selected from those commonly produced by commercially available electrofishing equipment. Electrical treatments included uninterrupted direct current (DC), and 110, 60, 30, 20, and 15 Hz square-pulsed DC. Pulse durations were fixed at 1 ms. All electrical variables were measured within the energized field with a Tektronix THS720A oscilloscope (Tektronix, Inc., Oregon).

Voltage applied to the electrical circuit may be measured as peak or root-mean-square (RMS). Peak voltage measures maximum amplitude attained by a pulse, while RMS quantifies the equivalent uninterrupted DC voltage applied by different forms of pulsed DC (Kane and Sternheim 1988). Both peak and RMS voltages were measured. However, because the pulses were square, RMS voltage was a fixed fraction of peak voltage. For DC and 110, 60, 30, 20, and 15 Hz pulsed DC with 1 ms pulses, RMS voltages were 1, 0.350, 0.261, 0.184, 0.150, and 0.128 of the peak voltages, respectively. Following Kolz and Reynolds (1989), peak voltage was used to calculate power density (i.e., peak power density); nevertheless, mean power density (i.e., power density computed based on RMS voltage) can be derived as the product of peak power density and the RMS fraction squared (e.g., for 110 HZ, 1 ms, RMS power density = 0.350^2 x peak power density).

Conductivity Levels

Various water conductivity levels were prepared by mixing well water (195 S cm^{-1}) with deionized water, or with sodium chloride (table salt). Specific conductivity

($C_s, \mu S \cdot cm^{-1}$) and ambient water temperature (T_w) were recorded with a YSI 30/10 FT meter (Yellow Springs Instruments, Ohio). The meter read C_s at specific temperature (T_s, 25° C). However, electrofishing success depends on ambient water conductivity at ambient water temperature. Ambient water conductivity (C_w) was estimated from specific conductivity, specific temperature, and ambient water temperature as (Reynolds 1996)

$$C_w = \frac{C_s}{1.02^{T_s - T_w}} \quad (3)$$

Test Tank and Power Source

All testing was conducted indoors in a polyethylene tank measuring 2.0 m long, 1.5 m wide, and 1 .0 m deep. The tank was filled to a depth of 10 cm with well water. The cross sectional profile of the tank was equipped with two, 1.6 cm thick aluminum plate electrodes positioned near both ends of the tank, perpendicular to the longitudinal axis of the tank. Electricity was supplied to the plates via a Smith-Root 15-D POW unit (Smith-Root, Inc., Washington) modified to allow continuous rather than discrete voltage control, and equipped with additional smoothing capacitors to eliminate spikes and reduce ripples at the peak of the pulses (i.e., ripples averaged $\pm$ 6% of the amplitude). Conditions within the tank produced a homogeneous electrical field with a constant voltage gradient.

Test Fish

Channel catfish *Ictalurus punctatus* measuring 27-35 cm total length were used in all tests. This species was used because specimens were readily available from the Mississippi State University Aquaculture Center, where a laboratory was assembled and maintained. Concurrent research to identify immobilization thresholds of other species has shown that channel catfish do not exhibit extraordinary behavioral responses and immobilization thresholds (Dolan 2001). Prior to testing, fish were seined from earthen ponds and held in concrete raceways for at least two weeks, and maintained in good condition on a diet of artificial food. During testing, fish were transferred one at a time to the test tank and confined in the area between the two electrodes. After allowing 3-10 s for the fish to orient, the current was switched on when the fish oriented perpendicular to the electrodes. Treatment fish were exposed to voltages incrementing from near zero to levels higher than those needed to immobilize them within 3 s. The immobilization response was recorded as 0 for no immobilization and 1 if the fish was immobilized. A range of 10-35 fish was used per water conductivity level, depending on ease of identifying immobilization threshold. The reactions of each fish were observed and recorded, but were also videotaped via a camera positioned over the tank, allowing review of responses to verify the accuracy of live observations. After testing, fish were transferred to a holding tank, and later released into an earthen pond.

Goodness of Fit

For each electrical treatment and C_w level, the independent variable peak voltage was regressed on the dependent binary immobilization response using logistic regression. The resulting logistic models were used to predict the voltage level required for a 0.95 probability of immobilization (V). To test whether Kolz's model adequately represented observed responses in a range of C_w, P_w in equation 1 was substituted with equation 2 to solve for V

$$V = d\sqrt{\frac{P_f\left(1+\frac{C_f}{C_w}\right)^2}{4C_f}} \qquad (4)$$

Equation 4 was then fit according to electrical treatment to the V and C_w pairs using a nonlinear regression with a multivariate secant iterative method (NONLIN procedure; SAS 1996). Goodness of fit for Kolz's model was assessed by examining the magnitude and distribution of the residuals generated by fitting equation 4 to the empirical data. Magnitude of residuals was indexed with an R^2 statistic computed as $1\text{-}(y\text{-})^2 \cdot (y\text{-}\bar{y})^{-2}$ (model 1 of Kvalseth 1985).

Suitability of an Average Fish Conductivity

This assessment evaluated the effect of misspecification of C_f on the variability of the right-side quotient in equation 1. That quotient may be interpreted as a multiplier for constant power when equation 1 is rearranged to solve for P_w

$$P_w = P_f \frac{\left(1+\frac{C_f}{C_w}\right)^2}{4\frac{C_f}{C_w}} \tag{5}$$

In equation 5, when $C_f = C_w$ the multiplier is 1 because the power density in the fish is the same as in the water, but when $C_f \neq C_w$ the multiplier is >1. This analysis examined how the multiplier changed relative to C_f for a range of C_w values increasing from 25 to 1000 $\mu S \cdot cm^{-1}$. C_f was evaluated for high and low values selected from reports in the literature and from values derived by fitting equation 4. Thus, to assess suitability of an average C_f, this analysis compared the deviation of the multiplier caused by a potential error in specifying C_f.

Results

Objective 1 – Adequacy of Kolz's Model

In all, the responses of 1019 channel catfish were included in these tests. Ambient water conductivity levels ranged from 12 to 1030 $\mu S \cdot cm^{-1}$, and ambient water temperatures from 14 to 21° C. Voltages applied ranged from 3 to 608 V. V levels estimated with logistic regression ranged from 10 to 525 (Figure 1)

Values of V were related inversely and curvilinearly to ambient water conductivity (Figure 1). The power density that had to be transferred into the test fish to achieve immobilization (P_f) ranged from 15 to 1,089 $\mu W \cdot cm^{-3}$. P_f values were lowest for pulsed DC 60 and 110 Hz, suggesting these settings required the lowest power densities and voltages to immobilize the test channel catfish. P_f values increased dramatically as pulse frequency decreased, to a high of 1,089 for pulsed DC 15 Hz. Derived fish conductivity values ranged from 89 to 162 $\mu S \cdot cm^{-1}$, and exhibited no obvious trend relative to electrical setting. Equation 4 adequately described the observed relation between C_w and V, as indexed by high R^2 values that ranged from 0.92 to 0.99, and by relatively narrow 95% confidence limits around the P_f and C_f parameters (Figure 1). Residual plots showed no anomalous patterns.

Suitability of an Average Fish Conductivity

The literature search produced few estimates of fish conductivity. Estimates for various fish species were reported by Haskell (1954; 667 $\mu S \cdot cm^{-1}$), Whitney and Pierce (1957; 787-1025 $\mu S \cdot cm^{-1}$), Monan and Engstrom (1963; 505-1266 $\mu S \cdot cm^{-1}$), and Sternin et al. (1972; 319-3571 $\mu S \cdot cm^{-1}$). These estimates were made by measuring differences in resistance of water with and without fish. Another source of fish conductivity values was the TOBEC literature. TOBEC (total body electrical conductivity) has been applied to various fish species to measure proximate composition (e.g., Brown et al. 1993; Bai et al. 1994; Barziza and Gatlin, 2000), but it generates a unit-less index of body conductivity. The preceding measurements were disregarded because they estimated conductivity of a carcass, not in reference to behavioral responses in the context of power transfer concepts.

Some estimates of effective fish conductivity were available. Kolz and Reynolds (1989) measured effective conductivity of 6-9 cm goldfish *Carassius auratus* in laboratory tanks. Conductivity of fish was measured at "stunned immobility" defined as immediate loss of equilibrium, in contrast to immobilization within 3 s as defined in this study. Conductivity was 83 $\mu S \cdot cm^{-1}$ for fish exposed to DC, 156 for those exposed to 60 Hz AC, and 145, 160, and 137 for those exposed to 50 Hz pulsed DC with 2, 5, and 10 ms pulse widths, respectively. Jesien and Hocutt (1990) conducted similar testing with 18-21 cm channel catfish with various pulsed DC and AC settings, but included only three water conductivities, not enough to properly fit equation 4. Liu (1990) reported 50 Hz AC voltage gradients required to elicit a flight response in silver carp *Hypophthalmichthys molitrix* and bighead carp relative to seven water conductivities. Equation 4 adequately fit their data ($R^2 \geq 0.89$) and identified C_f values of 56 S cm^{-1} for bighead carp and 96 S cm^{-1} for silver carp. Given these distributions and those measured in our study, effective fish conductivity values ranging from 75 to 175 were considered in the multiplier of equation 5.

Over C_w the distribution of the multiplier was characterized by inverse parabolas that bottomed at 1 when C_w equalled C_f (Figure 2). The vertical distance between the parabolas indicates the potential error in specifying a multiplier. The error increased as

C_w values diverged from the 100-150 span. The $C_f = 75\ \mu S \cdot cm^{-1}$ and $C_f = 175\ \mu S \cdot cm^{-1}$ parabolas intersected at $C_w = 115\ \mu S \cdot cm^{-1}$, corresponding to a C_f that divided the distance between the parabolas roughly in half. Thus, in practical applications an average C_f of about 115 would result in the least multiplier error forced by a mistaken C_f. If C_f = 115 was used in equation 5 to estimate P_w for uniform electrofishing over waters with 100 and 400 $\mu S \cdot cm^{-1}$, the multipliers would be 1.005 and 1.441, respectively. However, if C_f was mistaken and the true value was 175, the multipliers would be 1.080 and 1.181 (Table 1). The effect of such an error on P_w would be $-0.075P_f$ and $0.260P_f$, and on voltage gradient $-0.037\left(P_f \cdot 100^{-1}\right)^{0.5}$ and $0.114\left(P_f \cdot 400^{-1}\right)^{0.5}$.

Discussion

Kolz's (1989) model adequately fitted the empirical C_w and V matched pairs, suggesting it can help standardize electrofishing. The R^2 values for the regressions were higher than 0.95, except for pulsed DC 15 Hz that had a 0.92 value. With this setting, fish exhibited a vigorous forced swimming behavior that made it hard to assert whether the fish had been immobilized within 3 s, even after reviewing recorded videos. This difficulty likely reduced the accuracy and precision of observations. Additional but trivial variability could have been introduced by experimental temperatures, which ranged 7°C, and may influence fish conductivity and their reaction thresholds (Whitney and Pierce 1957). Overall, variability around regression parameters was minimal and composed of experimental error rather than model lack of fit.

In this study, immobilization within 3 s was chosen as the reaction to recognize a threshold response to electricity. Conceivably, equivalent results may be obtained by choosing other reactions (e.g., fright response, twitch response, immobilization within 1 s) that may have different thresholds. Working with other reactions would affect the P_f parameter in equation 4 (e.g., lower P_f for twitch response, or raise P_f for immobilization within 1 s), but should not influence the C_f parameter. In this regard, Kolz and Reynolds (1989) documented that estimates of C_f made at two threshold responses were similar and deviated mainly due to measurement error.

Values of P_f ordered relative to pulse frequency, but derived values of C_f did not. The amount of peak power needed to immobilize fish was low for high-frequency settings and increased as frequency decreased. This effect may be attributed to longer off time associated with low-frequency waveforms that allow muscles more time to relax before stimulating them with the next pulse of electricity, and thus more time or higher peak power density is required to immobilize fish (Vibert 1967; Bird and Cowx 1990). This pattern does not account for DC's P_f that was intermediate between pulsed DC 20 and 30 Hz. However, the mechanism that produces immobilization is thought to be different for DC. The fluctuating current of pulsed DC reportedly produces stimulations of nervous fibers that lead to immobilization via cramping of muscles, whereas the continuous current of DC either inhibits or overexcites body cells and muscle fibres, but does not affect the nerve fibres (Lamarque 1967; Vibert 1967). An ordering was also expected for C_f given that measurement of effective conductivity integrates the response of the nervous system, which may respond differently to varied pulse patterns. The absence of such progression may reflect the small range in C_f and the inability to measure C_f more precisely. Kolz and Reynolds (1989) also found no progression in C_f for fish exposed to pulsed DC 50 Hz with 2, 5, and 10 ms pulse widths, but like in our study, found that C_f was lower for DC than pulsed DC treatments.

The question of whether average fish conductivity may be assumed to partially standardize electrofishing can be considered by evaluating the effect of an erroneous C_f on voltage gradient. The effect of a misestimated C_f on voltage gradient resulted in $\leq$5% error in waters with conductivities between 90 and 225, $\leq$10% in conductivities between 60 and 350, and <25% in conductivities between 20 and 1000. Most electrofishing is conducted in waters with 100-200 $\mu S \cdot cm^{-1}$ conductivity (Lazauski and Malvestuto 1990). Moreover, in a typical electrofishing field, voltage gradient ranges from 0.01 to 2 Vcm^{-1} (Kolz 1993), and a range of 0.1 to 1 Vcm^{-1} is often recommended for effective electrofishing (e.g., Vibert 1963; Lamarque 1967; Reynolds 1996). Relative to this variation, the effect of a misestimated C_f was small.

Kolz's power transfer model adequately predicted power levels required to elicit immobilization of channel catfish over a wide range of water conductivities. The model can help standardize electrofishing by allowing use of a standard power over space and time, reducing estimation error due to inconsistent application of electrical power. Burkhardt and Gutreuter (1995) reported that standardizing power improved consistency of electrofishing catch rates by about 15%. Standardization of power requires knowledge of fish effective conductivity, which likely varies relative to size, species, and season. Nevertheless, in this study use of a standard 115 $\mu S \cdot cm^{-1}$ effective conductivity introduced relatively little error in the estimation of standard power. This standard, however, was a middle value representing the few available estimates, and so more estimates of fish conductivity are needed. Application of Kolz's model cannot solve all standardization problems associated with electrofishing. Other variables that are likely to have a large effect on catch consistency are the shape and distribution of the power density field in the water, and fish size. Several authors have considered methods for standardizing electric fields (e.g., Novotny 1990; Kolz 1993) and for accounting for size selectivity (e.g., Buettiker 1992; Anderson 1995).

Standardization of electrofishing can help reduce variability of survey data, and potentially reduce injury to fish. With no standardization, differences among collections can be partially attributed to disparities in electrofishing efficiency, and not solely to disparities in fish assemblages. Adoption of a standard power transfer can help reduce the variability in electrofishing catches. Moreover, electrofishing can have detrimental effects on individual fish (Nielsen 1998). Because adverse effects can often be traced back to exposure to excessive power levels (Snyder 1995), standardization of power transferred to fish can also help minimize injury and mortality. Because electrofishing is an active capture method, complete standardization is probably impossible, but standardization of controllable variables can minimize bias and variability.

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Table 1. Effect of erroneously stipulating fish conductivity (C_f; $\mu S \cdot cm^{-1}$) on peak power applied to water (P_w; $W\ cm^{-3}$) and voltage gradient (Vcm^{-2}) for various water conductivities (C_w). C_f is stipulated as 115, but the true value is assumed to be 175. P_f = peak power that must be transferred into the fish to achieve immobilization or other threshold.

C_w	Q_{115}[a]	Q_{175}[a]	P_w error[b]	Voltage gradient error[c]
25	1.704	2.286	$-0.582P_f$	$-0.206\left(P_f \cdot 25^{-1}\right)^{0.5}$
50	1.184	1.446	$-0.262P_f$	$-0.115\left(P_f \cdot 50^{-1}\right)^{0.5}$
100	1.005	1.080	$-0.075P_f$	$-0.037\left(P_f \cdot 100^{-1}\right)^{0.5}$
200	1.076	1.004	$0.072P_f$	$0.036\left(P_f \cdot 200^{-1}\right)^{0.5}$
400	1.441	1.181	$0.260P_f$	$0.114\left(P_f \cdot 400^{-1}\right)^{0.5}$
600	1.852	1.430	$0.422P_f$	$0.164\left(P_f \cdot 600^{-1}\right)^{0.5}$
800	2.275	1.698	$0.577P_f$	$0.205\left(P_f \cdot 800^{-1}\right)^{0.5}$
1000	2.703	1.972	$0.731P_f$	$0.239\left(P_f \cdot 1000^{-1}\right)^{0.5}$

[a] $Q = (1+C_f C_w^{-1})^2\ [4(C_f C_w^{-1})]^{-1}$

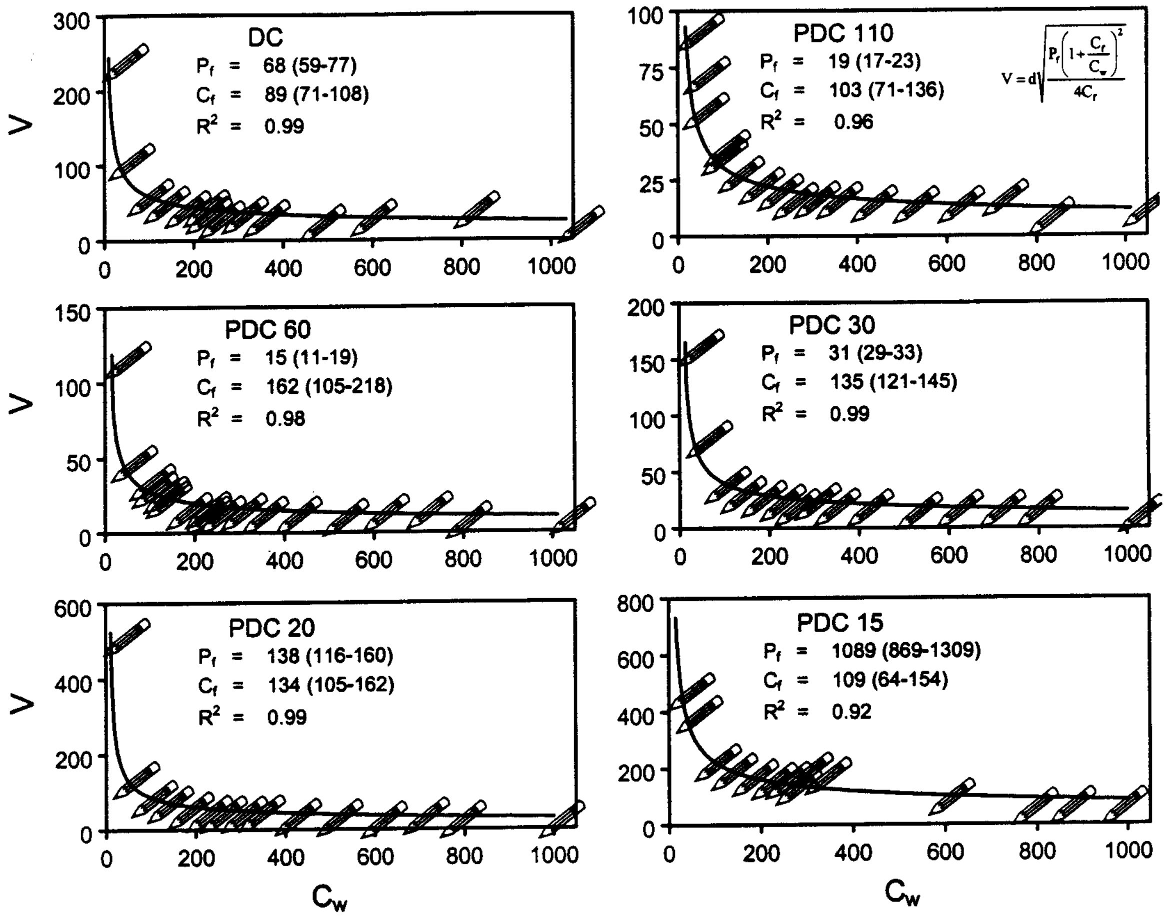
[b] error = $(Q_{115} - Q_{175})P_f$

[c] error = $(Q_{115}^{0.5} - Q_{175}^{0.5})\ (P_f\, C_w^{-1})^{0.5}$

Figure Captions

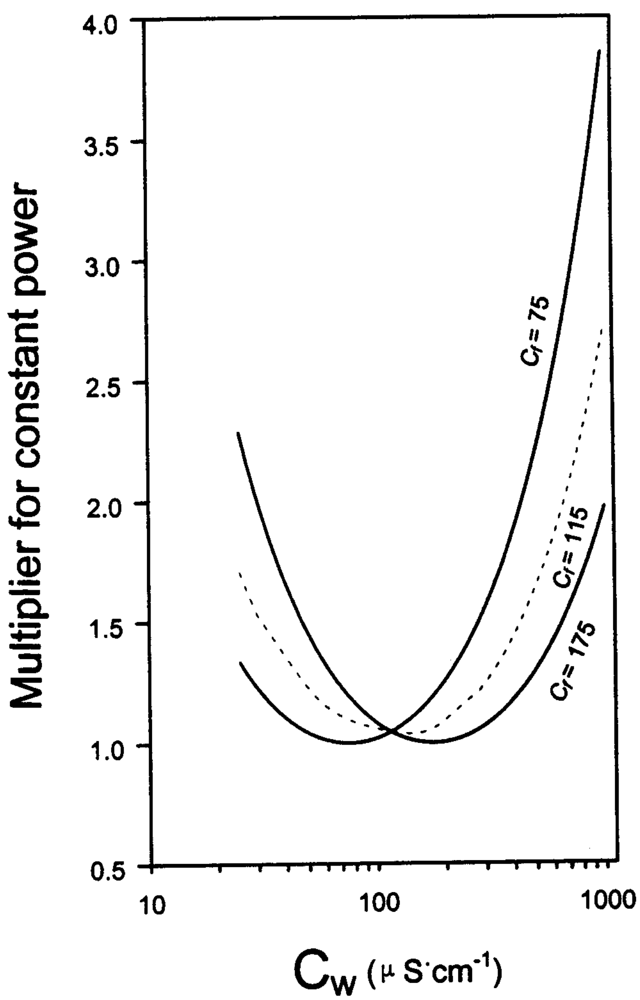
Figure 1. Relation between the voltage (V) needed to immobilize 95% of individuals and conductivity of water (C_w; $\mu S \cdot cm^{-1}$) for DC electrofishing, and 5 pulsed DC settings (PDC, Hz). The non-linear model fit to the data (equation 4) is given in the top panel. P_w = power density applied to the water, and P_f = portion of P_w that actually is transferred into the fish. Values in parentheses represent 95% confidence limits.

Figure 2. Relation between the multiplier for constant power density (right side quotient in equation 1) and conductivity of water (C_w). The multiplier was calculated for fish conductivity (C_f) of 75 and 175. A C_f of 115 was found to split the two curves about evenly.



DC
P_f = 68 (59-77)
C_f = 89 (71-108)
R^2 = 0.99
PDC 110
P_f = 19 (17-23)
C_f = 103 (71-136)
R^2 = 0.96
$V = d\sqrt{\frac{P_f\left(1+\frac{C_f}{C_w}\right)^2}{4C_f}}$
PDC 60
P_f = 15 (11-19)
C_f = 162 (105-218)
R^2 = 0.98
PDC 30
P_f = 31 (29-33)
C_f = 135 (121-145)
R^2 = 0.99
PDC 20
P_f = 138 (116-160)
C_f = 134 (105-162)
R^2 = 0.99
PDC 15
P_f = 1089 (869-1309)
C_f = 109 (64-154)
R^2 = 0.92
V
C_w

Figure 1.



4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Multiplier for constant power
$C_f = 75$
$C_f = 115$
$C_f = 175$
10
100
1000
C_W (μ S·cm⁻¹)

Figure 2.

Electrofishing Crappie: Electrical Settings Influence Immobilization Efficiency, Injury, and Mortality

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Abstract.-- Continuous direct current (DC) and pulsed DC (PDC) of varying frequency and pulse duration, are commonly used to immobilize and collect crappies *Pomoxis* spp. in freshwater. However, little information is available about minimum thresholds required for immobilization relative to electrical settings, and how these relate to incidence of injury and mortality. We investigated the effect of increasing power densities on immobilization, injury, and mortality of black crappie *Pomoxis nigromaculatus* (average TL = 154 mm) treated with DC and various PDC settings. Forced swimming toward the electrodes was observed in crappies exposed to DC, but less so for fish treated with PDC. Minimum peak power densities required to immobilize crappies ranged from 0.10 to 6.5 mW/cm^3, and depended on pulse frequency and duration. Incidence of hemorrhage ranged from 0 to 50% and spinal injury from 9 to 45%. However, severity of injury also depended on pulse frequency and duration. No fish suffered mortality at or below the immobilization thresholds, but mortality ranged from 0 to 15% above the thresholds. Mortality was observed with PDC 15 Hz settings only. Fish that were tetanized following electrical treatment were more prone to injury than those that exhibited narcosis.

Electrofishing is an efficient, commonly used method for collecting fish (Simpson and Reynolds 1977; Steinmetz 1990; Vaux et al. 2000). Fish captured via electrofishing are frequently released after sampling, so it is critical that fish survive and behave normally after release (Schneider 1992). Minimizing electrofishing-induced injury and mortality is especially important when studying populations through mark-recapture methods because death in tagged fish could lead to inflated population estimates or deflated exploitation estimates. Furthermore, reducing injury and mortality in fish may be important to Institutional Animal Care and Use Committees (IACUCs) as they evaluate the use of electrofishing for research.

Electrofishing-induced injuries in fish typically include tissue hemorrhage, spinal injury, and mortality (Reynolds 1996). Spinal injury may consist of fracture, and misalignment or compression of the vertebral column. Hemorrhage and bruising due to neural injury and dispersion of melanophores often accompanies spinal injury. These injuries can lead to immediate or delayed mortality (Bardygula-Nonn et al. 1995; Habera et al. 1996), which may occur within a few minutes or a few hours (Reynolds 1996). However, injuries are not always lethal or debilitating and they often heal, although behavior, health, growth, reproduction, and ultimately survival may be handicapped (Spencer 1967; Hudy 1985; Schill and Elle 2000).

The type of electric current used during electrofishing affects the incidence of injury and mortality. Alternating current (AC) is considered the most injurious, direct current (DC) the least injurious, and pulsed DC (PDC) intermediate to AC and DC (Hauck 1949; Lamarque 1990; Reynolds 1996). In experiments on brook trout *Salvelinus fontinalis*, brown trout *Salmo trutta*, and rainbow trout *Oncorhynchus mykiss* AC accounted for 11% mortality versus 2% in fish treated with DC (Pratt 1955). Similarly, AC caused 4% mortality in rainbow trout as compared to <1% for PDC, and 0% for DC (Taylor et al. 1957). For rainbow trout treated with various PDC settings, incidence of spinal injury ranged 3-62% and increased with pulse frequencies ranging 15-512 Hz (Sharber et al. 1994). In another study, PDC caused a greater incidence of injury in juvenile rainbow trout than DC, although injuries caused by PDC were less severe (Ainslie et al. 1998). Bluegill *Lepomis macrochirus* suffered up to 12% mortality with AC and up to 2% with DC (Spencer 1967).

Crappies *Pomoxis* spp. are the most harvested species in recreational fisheries in many regions of the country (Miranda 1999). Populations are often intensively managed to ensure the health of the fishery. In addition to other sampling methods (e.g., trap netting, gill netting, and trawling), electrofishing has been used to collect crappies (e.g., Maceina and Stimpert 1998; Maceina et al. 1998). Few studies have addressed injury to warmwater fish caused by electrofishing, and none have addressed crappies. Thus, no guidelines exist as to what electrical settings and voltages result in successful immobilization of crappies, but no or low injury and mortality. Also, injury and mortality in crappies that are by-catch of electrofishing surveys targeted at other species with similar habitat requirements (e.g., largemouth bass *Micropterus salmoides*) may be a concern. This lack of information encouraged us to test various electrical settings to identify immobilization thresholds for black crappie, to estimate extent of injury and mortality, to find methods for minimizing injuries while maximizing immobilization efficiency, and to suggest ways in which to standardize crappie electrofishing techniques.

Methods

Electrofishing research was conducted under controlled laboratory conditions at the Eastern Unit of the National Warmwater Aquaculture Center located at Mississippi State University. Experimentation as performed in a polyethylene plastic tank measuring 2.0 m long, 1.5 m wide, and 1.0 m deep. The tank was filled with well water to a depth of 10 cm. Two, 1.6 cm thick aluminum plate electrodes were positioned in the tank, perpendicular to the longitudinal axis of the tank, and spaced 65 cm apart. Conditions within the tank produced a homogeneous electrical field with a constant voltage gradient. During a treatment, a single fish was placed in the area between the two electrodes, and the electricity switched on when the fish oriented perpendicular to the electrodes. The reactions of individual fish were visually observed and documented via a video camera positioned over the tank, allowing review of responses to check the accuracy of observations.

The sampling design for this study was simple random sampling (i.e., fish were selected at random for treatment) nested within a sampling for modeling experimental design. Sample size was dictated by the amount of data needed to effectively model relationships (e.g., relation between an electrical measurement and immobilization), and thus, was variable. Seven electrical treatments consisting of a combination of pulse frequency and duration were considered (Figure 1). These treatments were selected from those commonly used in electrofishing and most readily available in commercial electrofishers. Settings considered were no pulse (DC), and PDC at 15, 60, and 110 Hz. Pulse durations evaluated for PDC were 1 and 6 ms for 110 Hz; 1 ms for 60 Hz; and 1, 4, and 6 ms for 15 Hz. Electricity was supplied to the plates via a Smith Root 15-D POW backpack electrofisher modified to allow continuous rather than discrete voltage control. Electrical characteristics between the plates were measured with a Tektronix THS720A oscilloscope (Tektronix, Inc., Oregon).

Test black crappie *P. nigromaculatus* averaged 154 mm total length (SD = 15) and 52 g total weight (SD = 17). Fish had been reared indoors for use in culture experiments. All fish had been fed the same diet, had not been previously exposed to electroshock, and had not received any other treatment that could have influenced their condition. Prior to experimentation, fish were held in concrete raceways for 48 h. A single fish was then captured at random with a dip net from the acclimation raceway and placed in the test tank with the electric current switched off. After allowing 3-10 s for the fish to orient, the current was switched on for 15 s. A total of 24-31 treatment fish and 5-6 control fish (i.e., no electricity applied) were used for each of seven experiments conducted over a six-week period from October to November 1999. Fish were exposed to power densities that ranged in equal increments from zero to the highest level allowed by the test equipment (1 fish per test power density). At each power density we recorded whether the test fish was immobilized within the first 3 s, and whether test fish exhibited narcosis or tetany by the completion of the 15 s period. The 3 and 15 s time periods were selected by estimating the amount of time required for fish escape from the electrical field and the maximum amount of time that a fish would be exposed to electricity in an actual field setting, respectively. Power density (P_w) is a function of conductivity (C_w, S/cm) and peak voltage gradient and was computed as (Reynolds 1996)

$$P_w = C_w \cdot \left(\frac{V_{pk}}{d} \right)^2 \qquad (1)$$

where V_{pk} is peak voltage and d is the distance between the electrodes (i.e., 65 cm). The P_w was incremented by raising voltage at a nearly constant conductivity that varied only due to small changes in temperature that might have taken place during the 1-2 h treatment period. Fish were treated only once, transferred to separate aerated 38 L holding tanks, and held for 18 h to allow hemorrhage to develop and for determination of short-term mortality.

Test conditions (temperature and conductivity) were measured with a YSI 30/10 FT meter (Yellow Springs Instruments, Ohio). The meter read specific conductivity (C_s) at a specific temperature (T_s) of 25° C. However, electrofishing success depends on conductivity at ambient water temperature (T_w). Ambient conductivity (C_w) was estimated from C_s, T_s, and T_w as (Reynolds 1996)

$$C_w = \frac{C_s}{1.02^{T_s - T_w}} \qquad (2)$$

Following the 18 h holding period, all fish were euthanized in a solution of 100 mg/L MS-222. Spinal injury was evaluated in a random subsample of treatment and control fish (Table 1) after preservation in formalin and subsequent clearing and staining according to Taylor and Van Dyke (1985). The remaining fish in the sample were necropsied. Necropsy included filleting the length of the body just posterior to the pectoral fins, along the rays and spine, to the caudal peduncle. For reference, digital photographs of all filleted fish (lateral view) were taken. Spinal injury and tissue hemorrhage were scored into an injury and no injury binary categorization.

Data Analyses

Immobilization, hemorrhage, spinal injury, and mortality data were analyzed using logistic regression (SAS Institute 1996). The independent variables for these analyses were power density (ratio scale) and electrical setting (nominal scale). Transformations of power density were used when pertinent to improve linearity, homogeneity of variances, and thus, overall fit of the resulting models. The dependent variable was the binary level of behavioral response (i.e., 0 = no immobilization, 1 = immobilization; 0 = no injury, 1 = injury; 0 = alive, 1 = dead). The power density required for a 0.95 probability ($P_{w,0.95}$) of immobilization was predicted from the logistic regression model. The 0.95 probability level was selected because it is associated with high immobilization efficiency (which may translate into increased capture efficiency), but the likelihood of producing as much injury as with power densities associated with greater probability levels is decreased. Analyses of injury and mortality data were limited to fish treated above $P_{w,0.95}$. Fish treated below $P_{w,0.95}$ were excluded because electrofishing is commonly conducted with power densities high enough to induce immobilization, and because including them artificially reduced overall levels of injury and mortality. The incidence of hemorrhage, spinal injury, and mortality in fish treated with power densities above $P_{w,0.95}$ were compared across electrical settings with multiple linear contrasts (SAS Institute 1996). Observations about the behavior displayed by fish at the conclusion of the 15 s period (i.e., narcosis or tetany) were used to categorize injury. Fisher's exact tests (SAS Institute 1996) were applied to test if incidence of hemorrhage, spinal injury, and mortality differed between the narcosis and tetany endpoints.

Significance for statistical tests involving immobilization was established at α = 0.05, and significance of tests involving injury and mortality at α = 0.20. For immobilization, limiting the probability of a Type I error (detecting differences when they do not exist) was important to avoid unwarranted claims regarding differential effectiveness over electrical settings. For injury and mortality, the probability of making a Type I error was relaxed to err on the side of caution due to the nature of the effect being tested. Of utmost concern was the probability of making a Type II error (failing to detect differences in injury and mortality when they do exist), which was reduced with a greater alpha level.

Results

A total of 221 fish ranging from 119-200 mm total length and 21-120 g body weight were included in these tests. Voltages were applied at conductivities of 191-193 μS/cm and temperatures of 20-25° C and ranged from 0-1,160 V, translating into voltage gradients of 0-18 V/cm and peak power densities of 0 to 61 mW/cm^3. Logistic regression models indicated the probability of immobilization was directly related to peak power density ($P < 0.01$), but the effect of power density differed among electrical settings ($P <$

0.01; Table 2). Logistic regression values for the electrical settings indicated the PDC 15 Hz settings (particularly PDC 15 Hz, 1ms) required the greatest peak power densities to immobilize fish (a large negative electrical setting value, β_e, in Table 2 suggests a high peak power density is needed to influence the effect). Values of $P_{w,0.95}$ ranged from 0.10 (PDC 60 Hz, 1ms and PDC 110Hz, 1ms) to 6.5 mW/cm^3 (PDC 15 Hz, 1 ms; Figure 2A). Mid-level to high-frequency settings (PDC 60 and 110 Hz) exhibited the least $P_{w,0.95}$, while low-frequency settings the greatest. The $P_{w,0.95}$ for DC was intermediate.

No injury or mortality was observed in control fish (Table 1). In treatment fish, all injuries occurred in the caudal peduncle region. Spinal injury usually consisted of the compression of 2-3 caudal vertebrae, without discernible fractures. Hemorrhages occurred at the functional hinge (joint that allows side to side movement of caudal peduncle) of the vertebral column, and ranged from 1-3 vertebrae in size. Mortality occurred at random intervals over the first 3 h of the 18 h holding period only.

Incidence of hemorrhage above $P_{w,0.95}$ averaged 23% and ranged from 0 (DC) to 50% (PDC 110 Hz, 1 ms) over electrical settings (Table 1; Figure 2B). The logistic regression model indicated the probability of hemorrhage was directly related to power density (P = 0.03), but the incidence of hemorrhage above $P_{w,0.95}$ was significantly different among electrical settings ($P < 0.01$; Table 2). Logistic regression values for the electrical settings indicated DC required the greatest peak power densities to induce hemorrhage, and PDC 110 and 60 Hz the least. For the PDC settings, hemorrhage appeared to decrease with decreasing frequency and pulse duration, but this trend could not be tested statistically because of the limited number of frequencies and pulse duration permutations included in this study.

Incidence of spinal injury above $P_{w,0.95}$ averaged 22% and ranged from 9% (DC) to 45% (PDC 110 Hz, 1 ms) over electrical settings (Table 1; Figure 2C). The logistic regression model indicated the probability of spinal injury was directly related to power density (P = 0.01). However, the effect of power density did not differ among electrical settings ($P > 0.20$; Table 2).

No mortality was observed above $P_{w,0.95}$ for the DC, PDC 110 Hz, PDC 60 Hz, and PDC 15 Hz, 1 ms electrical settings (Table 1). Mortality occurred in the PDC 15 Hz, 1 ms treatment (7%) and PDC 15 Hz, 4 ms treatment (15%) only. A logistic regression model like those developed for hemorrhage and spinal injury could not be fitted because of the low incidence of mortality.

Incidence of hemorrhage for fish narcotized after the 15 s treatment period was 4% (N =27) and differed significantly from the 28% (80) exhibited by fish that ended up tetanized ($P < 0.01$). Incidence of spinal injury for fish narcotized was 11% (N = 9) and did not differ significantly from the 23% (N = 64) exhibited by fish that were tetanized ($P = 0.67$). No mortality (N = 27) was observed in fish that exhibited narcosis, but 4% (N = 80) of fish that were tetanized died. However, this difference was not statistically significant ($P = 0.57$).

In addition to the immobilization, injury, and mortality observations summarized above, we made observations about the behavioral reactions of crappie treated with the various electrical settings. Because these observations were not conducive to statistical analyses, we provide a descriptive narrative instead. The DC setting caused fish to exhibit electrotaxis (forced swimming) towards the anode (positive electrode) before immobilization within 3 s; this attraction was conspicuous and occurred immediately upon electrification of the field. However, at high levels of DC (i.e., well above $P_{w,0.95}$) fish were immobilized instantly once the field was electrified, with no obvious forced swimming towards the anode. Attraction towards the anode was observed in a few fish treated with other settings, but was not as evident as with DC. Fish exposed to low-frequency settings (PDC 15 Hz) exhibited quivering or strong vibrations after immobilization (such behavior might have occurred before immobilization but could not be observed until fish stopped swimming). Moreover, quivering appeared to be more severe in treatments with short-pulse durations (i.e., PDC 15 Hz, 1 ms).

Discussion

Because fish responses to electrical fields vary with fish size, the $P_{w,0.95}$ values identified are size specific and projected to be lesser for larger crappies and greater for smaller ones; nevertheless, they provide approximate targets to standardize electrofishing over changing conductivities. A first step towards standardization may be to adjust voltage to homogenize power density over waters with diverse conductivities. To this end, equation 1 serves to adjust voltage output to compensate for a change in conductivity and maintain $P_{w,0.95}$ approximately constant. A second step towards standardization may be to adjust $P_{w,0.95}$ to compensate for the difference between water and fish conductivities. According to Kolz (1989) PD (thus, $P_{w,0.95}$) varies relative to water conductivity because the efficiency with which power transfers to the fish depends on the difference between the conductivity of the water and the effective conductivity of the fish (full transfer occurs when conductivities match; Kolz's equation 38 adjusts power transfer for unequal conductivities). Unfortunately, the effective conductivity of a fish changes with electrical setting (Kolz and Reynolds 1989) because fish are not directly wired and have a nervous system that reacts differently to diverse electrical stimuli. In addition, fish effective conductivity is likely to change with species, life stage, and other biological characteristics that affect chemical makeup, and thus, electrical conductance. Another limitation to standardizing power transferred to fish is the inability to create homogeneous electrical fields under field conditions (Reynolds 1996). Despite these limitations, efforts to standardize electrofishing to maintain relatively constant power have reduced inter-site variability in catch rates (Burkhardt and Gutreuter 1995).

The low-frequency electrical settings required greater $P_{w,0.95}$ to immobilize fish than DC or mid-level to high-frequency settings. Vibert (1967) speculated that differences in immobilization between high- and low-frequency electrical settings may be attributed to longer off-times associated with low-frequency settings. These pulse frequencies allow muscles more time to relax before stimulation with the next pulse of electricity, and thus, more time or greater peak power density may be required to immobilize fish. Conversely, DC and mid to high-frequency electrical settings stimulate fish muscles constantly or nearly so, limiting relaxation time and potentially leading to quicker cramping and immobilization at lesser peak power densities. A similar scenario may have led to our observations of increased $P_{w,0.95}$ for low-frequency treated fish in this study.

Traditionally, radiography has been used to evaluate spinal injury. However, the method of clearing and staining was used for evaluation of spinal injuries in this study. We felt that clearing and staining allowed us to more adequately assess spinal injury because fish skeletons could be observed from many different angles as opposed to only a single perspective as with radiography. Furthermore, clearing and staining allowed us to save the actual test specimens for further scrutiny of potential spinal injuries. The drawbacks of clearing and staining are that it is a more time consuming method than radiography, and nearly as costly.

Incidence of hemorrhage and spinal injury appeared to be linked to pulse frequency, with greater frequencies leading to more injuries and lesser frequencies or no pulsation to fewer injuries. The two PDC 110 Hz settings produced the most hemorrhages and spinal injuries. Similarly, Sharber et al. (1994) found that incidence of spinal injury increased with pulse frequency in rainbow trout, and hypothesized that more fish injuries occurred at high pulse frequencies because injuries were caused by myoclonic jerks associated with shock-induced seizures, and such seizures developed more rapidly at greater than lesser frequencies. These same mechanisms could be contributing to the high incidence of spinal injury and hemorrhage observed in black crappie treated with PDC 110 Hz settings.

Absence of a highly developed muscle mass may make crappies susceptible to hemorrhage and spinal injury. The species has evolved large dorsal and ventral fins and a laterally compressed body to facilitate maneuverability in their favored habitats, which include submerged structures and steep slopes (Pflieger 1997). This adaptation has led to a reduced muscle mass around the spinal column (Helfman et al.1997), a deficiency that may contribute to vulnerability to hemorrhage and spinal injury. Poorly developed lateral muscles offer little protection or cushion from the effects of anodic curvature, and severe contortions that result from this curvature may lead to injuries (Lamarque 1990). Black crappies were observed to contort at the functional hinge in the caudal peduncle, where hemorrhage and spinal injury were most prevalent.

Traditionally, electrofishing has been considered most effective when conducted with settings that have maximum tetanizing effects (Lamarque 1990). Our immobilization and injury observations cause us to question this approach. First, although immobilization was more instantaneous when applying electrical power at levels that tetanized fish, the benefits of electrotaxis recorded with DC were lost when fish were tetanized. Second, tetanized fish exhibited more injury, possibly as a result of severe muscle contractions that can result in tissue and bone damage (Lamarque 1990; Reynolds 1996). Narcosis and tetany endpoints depend on the nature and intensity of the electrical field, which can be manipulated by managing power density through reductions or increases in voltage or amperage (Reynolds 1996). Given that injury levels were reduced in fish that were narcotized but not tetanized, operating equipment to produce power densities that induce narcosis rather than tetany can reduce injury. Such an approach to electrofishing can facilitate collection applications by allowing control of electrical output through observation of fish behavior in the electrical field, rather than through in-water measurement of electrical variables. However, this observational approach would not address the standardization issues pursued by Burkhardt and Gutreuter (1995).

Mortality of black crappie treated with PDC 15 Hz may be attributed to various factors other than the injuries studied. In a study of rainbow trout, Taylor et al. (1957) concluded that mortality often appeared to result from factors that were not visible either grossly or microscopically. For example, the persistence of tetany after the interruption of current may prevent resumption of respiration, leading to suffocation and eventually death (Lamarque 1990). In addition, researchers have found that electrical current can alter blood constituents, and suggested that the stress associated with these changes may reduce survival (Barton and Grosh 1996; Barton and Dwyer 1997). We observed in our study that fish treated with PDC 15 Hz, unlike other electrical settings, vibrated or quivered vigorously. This vibration was consistent with the symptoms (twitches, jerks, and convulsions) of epileptic seizure described by Sharber and Black (1999), who stated that seizures could be induced in many vertebrates (including fish) by passage of electrical current through the brain. Epileptic seizures have been suggested as cause for gross physical injuries such as spinal injury (Sharber et al. 1994), and it follows that seizures may also result in less detectable injuries (i.e., organ, tissue, and cell damage) that may eventually lead to death. Such a scenario may have contributed to death of fish in this experiment. However, because little information exists regarding the effect of seizure on mortality, additional research is needed to identify the precise cause of death of black crappie exposed to low-frequency electrical pulses. In the meantime, it may be best to avoid the use of low-frequency electrofishing to minimize mortality of shocked black crappie. Also, it may be appropriate to exercise the same caution when electrofishing white crappie. Black crappie and white crappie frequently occupy the same habitats and, in most respects, have similar biology (Pflieger 1997).

In conclusion, our research showed that electrofishing with enough power to immobilize crappie resulted in injuries to the fish. High-frequency settings produced the greatest levels of injury, and low-frequency settings the greatest levels of mortality; whereas, DC produced the least injury and mortality. Additionally, black crappie exposed to DC often exhibited forced swimming towards the electrodes, a feature that may allow more efficient extraction of fish from cover or deep water. However, with the DC setting the current was on continuously, necessitating more power than with a PDC setting (greater mean $P_{w,0.95}$, Figure 2A), which may decrease the utility of DC electrofishing in low or high conductivity waters (Reynolds 1996). The PDC 60 Hz may be the next best alternative to DC when low power output is an issue. This setting requires less power because time on is reduced, while causing less injury and mortality than PDC settings with greater or lesser frequencies. The $P_{w,0.95}$ values identified provide approximate targets for electrifying black crappie; managing voltage output levels to induce narcosis and avoid tetany provides a suitable alternative to measuring power densities in water and power transferred to fish.

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Table 1. Incidence (%) of hemorrhage, spinal injury, and mortality in black crappie treated to power densities equal to $P_{w,0.95}$ or higher. No power was applied to the control fish. The values in parentheses represent sample size.

Electrical setting	Hemorrhage	Spinal injury	Mortality
DC	0 (14)	9 (11)	0 (14)
PDC 110 Hz, 6 ms	43 (14)	20 (10)	0 (14)
PDC 110 Hz, 1ms	50 (12)	45 (11)	0 (12)
PDC 60 Hz, 1ms	33 (15)	10 (10)	0 (15)
PDC 15 Hz, 6 ms	14 (14)	20 (10)	7 (13)
PDC 15 Hz, 4 ms	15 (13)	20 (10)	15 (14)
PDC 15 Hz, 1 ms	7 (14)	27 (11)	0 (15)
Controls	0 (16)	0 (20)	0 (16)

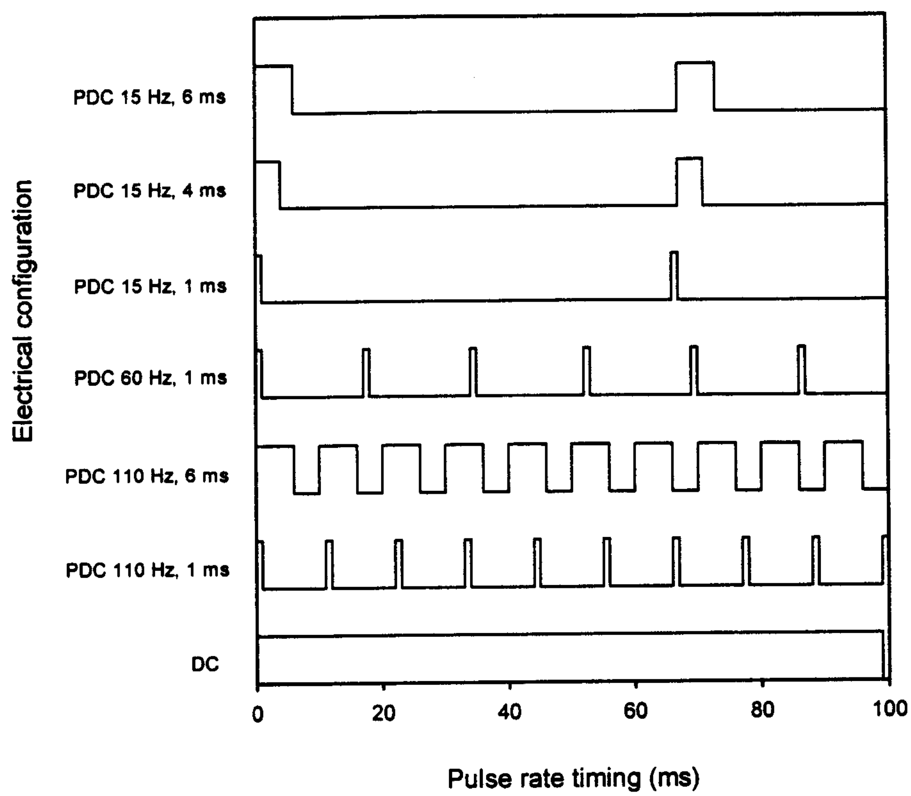
Table 2. Logistic regression models for predicting probability (p) of immobilization, hemorrhage, and spinal injury of black crappie according to electrical setting. Models are of the form $logit = (\beta_o + \beta_e) + \beta_1 x_i$ where β_o = intercept, β_e = electrical setting parameter, β_1 = slope, and $x_i = P_{w,0.95}$ (mW/cm^3). Different transformations of $P_{w,0.95}$ were used for immobilization, hemorrhage, and spinal injury. Example: the immobilization model for black crappie treated with DC becomes $logit = (12.9 - 3.97) + 4.26\,[\log_e(P_{w,0.95})]$. Logits were back transformed to p, $p = e^{(logit)}/[1 + e^{(logit)}]$. β_e within the same column that share a letter in common are not significantly different (pairwise comparisons were made at α = 0.05 for immobilization and α = 0.20 for injury).

Parameter		Immobilization	Hemorrhage	Spinal injury
β_o		12.9	-0.581	-3.20
β_1	$\log_e(P_{w,0.95})$	4.26		
	$P_{w,0.95}^{-1}$		-0.131	
	$P_{w,0.95}$			0.070
β_e	DC	-3.97 (zvu)	-25.5 (z)	0.682 (z)
	PDC 110 Hz, 6 ms	-2.50 (zxv)	0.565 (y)	1.49 (z)
	PDC 110 Hz, 1 ms	-0.202 (yxt)	0.711 (y)	1.75 (z)
	PDC 60 Hz, 1ms	0 (t)	0 (yw)	0 (z)
	PDC 15 Hz, 6 ms	-5.01 (u)	-1.20 (x)	0.864 (z)
	PDC 15 Hz, 4 ms	-5.02 (vu)	-1.10 (xw)	0.839 (z)
	PDC 15 Hz, 1 ms	-18.0 (w)	-2.33 (zx)	-0.347 (z)

Figure Captions

Figure 1. Pulse shape, width, and repetition pattern for seven experimental electrical settings.

Figure 2. (A) $P_{w,0.95}$ required to immobilize black crappie, (B) % hemorrhage above $P_{w,0.95}$, and (C) % spinal damage above $P_{w,0.95}$. In panel A, the values in parentheses represent mean $P_{w,0.95}$ (defined in methods section); in panels B and C the values in parentheses printed above observed injury represent predicted injury at $P_{w,0.95}$ (derived from equations in Table 2).



Electrical configuration
PDC 15 Hz, 6 ms
PDC 15 Hz, 4 ms
PDC 15 Hz, 1 ms
PDC 60 Hz, 1 ms
PDC 110 Hz, 6 ms
PDC 110 Hz, 1 ms
DC
0
20
40
60
80
100
Pulse rate timing (ms)

Figure 1.

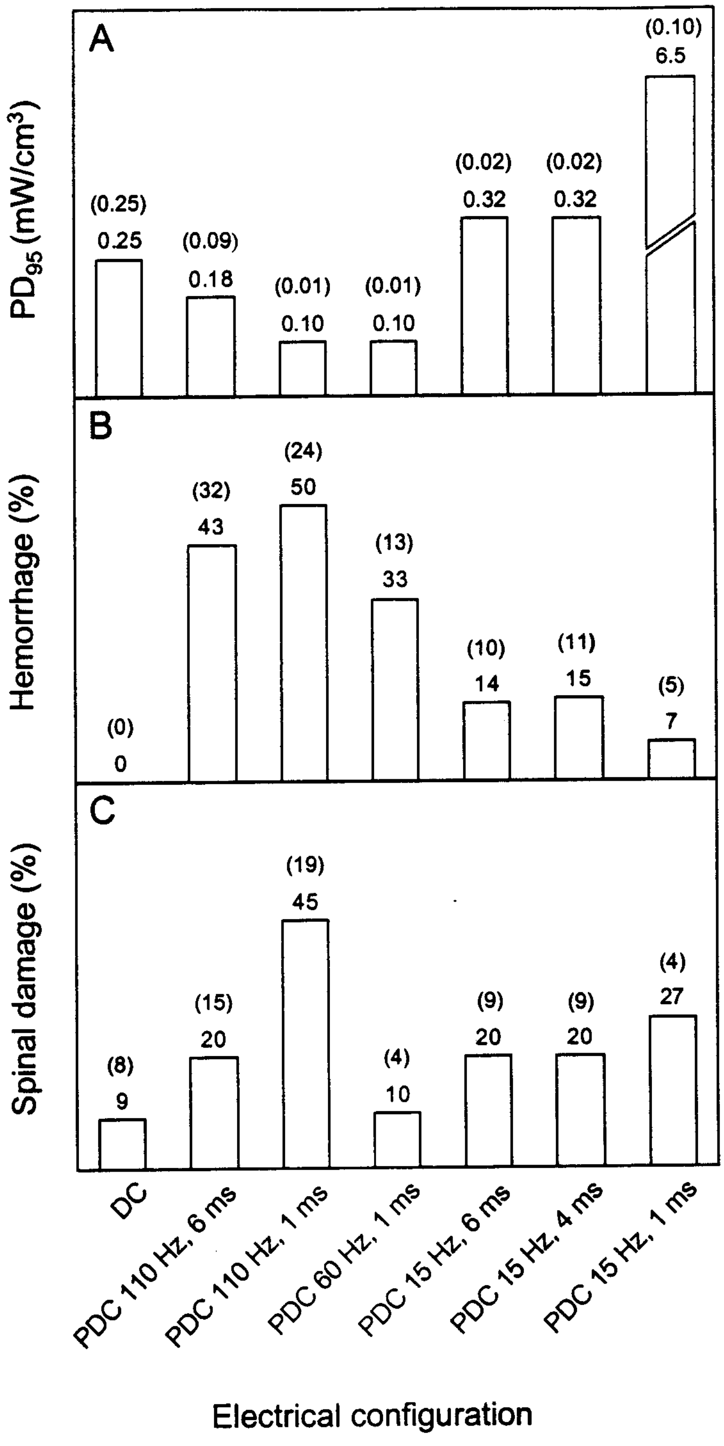

A
B
C
PD$_{95}$ (mW/cm^3)
Hemorrhage (%)
Spinal damage (%)
(0.25)
0.25
(0.09)
0.18
(0.01)
0.10
(0.01)
0.10
(0.02)
0.32
(0.02)
0.32
(0.10)
6.5
(0)
0
(32)
43
(24)
50
(13)
33
(10)
14
(11)
15
(5)
7
(8)
9
(15)
20
(19)
45
(4)
10
(9)
20
(9)
20
(4)
27
DC
PDC 110 Hz, 6 ms
PDC 110 Hz, 1 ms
PDC 60 Hz, 1 ms
PDC 15 Hz, 6 ms
PDC 15 Hz, 4 ms
PDC 15 Hz, 1 ms
Electrical configuration

Figure 2.

Histopathology of Fish Exposed to Electrofishing

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Abstract.— Under controlled laboratory conditions, bluegill *Lepomis macrochirus*, channel catfish *Ictalurus punctatus*, and largemouth bass *Micropterus salmoides* of various sizes were electrofished with continuous DC and various pulsed DC settings. Power densities applied ranged from zero (control) to levels high enough to tetanize and cause mortality to fish. Our objectives were to determine whether histopathology could reliably detect electrofishing-induced injuries, distinguish tissues most sensitive to the effect of electrofishing, and identify electrofishing modes that produced the least injury to fish tissues. Various lesion types were scored for each of eight types of tissue, including brain, gill, heart, spleen, liver, pancreas, posterior kidney, and muscle tissue. Most tissues showed few identifiable lesions, and few consistent histological changes were noted that were different from the non-electrofished controls. Histopathological examination of the controls demonstrated variability in the initial health status of the fish used in the study, and this variability may have masked subtle effects of the electric current exposure. Therefore, no clear trends between electrofishing and histopathology could be identified.

Injury to fish tissues by electrofishing has traditionally been assessed through macroscopic external and internal examinations (Lamarque 1990; Reynolds 1996). Bruises, brands, gill hemorrhages, and vertebral dislocations are the most common injuries manifested externally (Emery 1984; Lamarque 1990; Snyder 1995). External evidence of injury may also signal internal injuries (Horak and Klein 1967). However, except when particularly severe, internal injuries can only be detected by necropsy and X-ray (Sharber and Carothers 1988; Hollender and Carline 1994), or by clearing and staining (Taylor and Van Dyke 1985). Internal injuries can range from minor hemorrhages isolated in dorsal white muscle, to compression, distortion and fracture of one or more vertebrae accompanied by rupture of the dorsal aorta (Hauck 1949; Sharber and Carothers 1988; Thompson et al. 1997). Procedures and a scoring system have been developed for macroscopically evaluating the severity of electrofishing-induced internal injuries (Reynolds 1996). Moreover, electroshock was demonstrated to not adversely affect immune function or disease severity in spring chinook salmon (VanderKooi et al. 2001).

Alternatively, injury may be evaluated microscopically by identifying the presence of abnormal tissue. Histopathology is the study of disease progression at the microscopic level. Disease is classically defined as any condition deviating from a normal, healthy state. A microscopic scale permits evaluation of effects not evident through a macroscopic inspection, as well as examination of a larger diversity of tissues that can be collected simultaneously from a variety of organs in a single specimen (Fawcett 1994). Although histopathology has seldom or never been applied to the study of electrofishing-induced injury, it is often used in toxicology studies to assess injury hastened by exposure to toxic chemicals (Meyers and Hendricks 1985; Bucher and Hofer 1993).

Examination of fish tissue may help identify electrofishing modes that are least injurious to fish, determine the cause of death, and possibly identify injury mechanisms, or at least steer the researcher to potentially instructive sites for subsequent analyses. Furthermore, histopathological analyses may help provide a more complete and accurate description of the affects of electrofishing. Nevertheless, histopathology has not been previously applied to the study of electrofishing injury. Thus, our objectives were to determine whether histopathology could reliably detect electrofishing-induced injuries, distinguish tissues most sensitive to the effect of electrofishing, and identify electrofishing modes that produced the least injury to fish tissues.

Methods

Test Tank and Power Source

All testing was conducted from March 1999 to February 2000 under controlled conditions at the Mississippi State University Aquaculture Center, where a laboratory was assembled and maintained. Experimentation was performed in a polyethylene tank measuring 2.0 m long, 1.5 m wide, and 1.0 m deep. The tank was filled to a depth of 10 cm with well water. The cross sectional profile of the tank was equipped with two, 1.6 cm thick aluminum plate electrodes positioned 65 cm apart, perpendicular to the longitudinal axis of the tank. Electricity for most treatments was supplied to the tanks via a Smith-Root 15-D POW electrofisher (Smith-Root, Inc., Washington) modified to allow continuous rather than discrete voltage control, and equipped with additional smoothing capacitors to eliminate spikes and reduce ripples at the peak of pulses (i.e., ripples averaged ± 6% of the amplitude). A Coffelt Mark X electrofisher (Coffelt Manufacturing, Arizona) was used to apply a Coffelt trademark pulse train (see below). Conditions within the tank produced a homogeneous electrical field with a constant voltage gradient. Specific conductivity (C_s, μS/cm^{-1}) and ambient water temperature (T_w) were recorded with a YSI 30/10 FT meter (Yellow Springs Instruments, Ohio). The meter read C_s at specific temperature (T_s, 25° C). Ambient water conductivity (C_w) was estimated from specific conductivity, specific temperature, and ambient water temperature as (Reynolds 1996)

$$C_w = \frac{C_s}{1.02^{T_s - T_w}} \qquad (1)$$

Electrical Treatments

Six electrical treatments representative of pulse frequencies and periods commonly available in commercial electrofishing units were considered. These included continuous DC, pulsed DC 110 Hz, 6 ms, pulsed DC 110 Hz, 1 ms, pulsed DC 15 Hz, 6 ms, pulsed DC 15 Hz, 1 ms, and Coffelt's CPS™. The CPS™ delivers a fixed complex pulse pattern consisting of three 240 Hz, 2.6 ms square-pulses, each separated by 1.6 ms, and repeated 15 times/second.

Peak voltage (V_{pk}), pulse frequency, and pulse period (i.e., width) were measured within the energized field with a Tektronix THS720A oscilloscope (Tektronix, Inc., Oregon). Following Kolz and Reynolds (1989), V_{pk} was used to calculate power density (P_w) as

$$P_w = C_w \cdot \left(\frac{V_{pk}}{d} \right)^2 \qquad (2)$$

where d is the distance between the electrodes (i.e., 65 cm).

Test Fish

We applied the six electrical treatments to bluegill *Lepomis macrochirus*, channel catfish *Ictalurus punctatus*, and largemouth bass *Micropterus salmoides* of various sizes. These species were selected because they were readily available from the Mississippi State University Aquaculture Center or local commercial fish suppliers. Prior to testing, fish were seined from holding ponds, held in concrete raceways for at least 48 h, and maintained in good condition on a diet of live or artificial food, depending on the species.

During testing, fish were transferred one at a time to the test tank and confined in the area between the two electrodes. After allowing 3-10 s for the fish to orient, and when the fish was positioned perpendicular to the electrodes, the current was switched on for 15 s. As individuals, fish were treated once and to a single power density, but as a group, fish were exposed to power densities incrementing from zero to levels exceeding those needed to immobilize them within 3 s. Power density was incremented by raising voltage at a nearly constant conductivity that varied only due to small changes in temperature that might have occurred during the 1-2 h treatment period. The immobilization response (i.e., within 3 s) was recorded as 0 for no immobilization and 1 if the fish was immobilized. In addition, we recorded whether the test fish exhibited tetany by the completion of the 15-s period. The 3-s period estimated the time within which if the fish was not immobilized, it would likely escape the electrical field; the 15-s period estimated the maximum amount of time that a fish would be exposed to electricity in an actual field setting.

The number of fish tested per treatment ranged from 12 to 41, including 2-3 controls (i.e., no power density applied). All of these fish were used in conjunction with a larger study that evaluated immobilization thresholds; thus, sample size was dependent upon the number of individuals needed to identify immobilization thresholds. The reactions of each fish to the electricity were noted, but were also recorded via a video camera positioned over the tank, allowing review of responses to verify the accuracy of live observations. Following treatment, fish were transferred to separate aerated 38-L holding tanks, and held for 18 h for determination of short-term mortality.

Histopathology

After the 18 h holding period, fish were euthanized by an overdose of tricaine methane sulfonate, radiographed, and necropsied. Brain, gill, heart, spleen, liver, pancreas, posterior kidney, and muscle tissue samples were taken from each fish. These samples were placed in 10% phosphate buffered formalin for 24 h, and then dehydrated through gradient 25-95% ethanol series. Next, tissues were cut, trimmed, and placed into tissue cassettes. Tissues were then infiltrated and embedded in paraffin, sectioned at 5 μm thickness and stained with hematoxylin and eosin. Tissues were evaluated with the pathologist blinded to the treatment. Each tissue was viewed on an Olympus BH-2 microscope at 40x and 100x, and the presence and severity of lesions scored.

Several lesions were selected for evaluation, including necrosis, cellular changes, vascular changes, hemorrhage, and inflammation. Necrosis, or cell death, was scored 1 to 3 for focal, diffuse and extensive, respectively. Cellular change may be reflected as hypertrophy, hyperplasia, and nuclear condensation. Cellular hypertrophy is the enlargement of a cell usually resulting from loss of the cellular mechanisms controlling movements of solutes across the cell membrane. Hyperplasia is an increase in the number of a particular cell type, a condition that can occur due to sub-lethal irritation or acute inflammation. Nuclear condensation occurs when the cell nucleus shrinks and becomes smaller than usual. Cellular changes were scored 1 to 5 for mild hyperplasia, moderate hyperplasia, severe hyperplasia, hypertrophy and nuclear condensation, respectively. Vascular changes may be evidenced as congestions, aneurysms, thrombi, and infarction. Congestion is the presence of an excessive amount of blood and results from a sudden increase of blood flow, or from the inability of the tissue to effectively maintain normal blood flow. In congested tissues, accumulated blood remains within blood vessels. Aneurysms are localized abnormal swelling of a vessel, and can result from physical trauma or a weakened vessel wall. Thrombi are blood clots and occur when sufficient tissue injury has released the soluble factors that result in blood clot formation. An infarction is an area of tissue that is undergoing necrosis after the blood supply has been cut off, usually resulting from a thrombus. Vascular change was scored 1 to 6 for mild congestion, moderate congestion, severe congestion, aneurysm, thrombus, and infarction, respectively. Hemorrhage is blood or red blood cells present outside of blood vessels and suggests that vessel integrity has been compromised. Hemorrhage was scored 1 to 3 for focal, diffuse, and extensive, respectively. Inflammation is the tissue response to injury and in this study was differentiated as lymphocytic, granulocytic or monocytic referring to an influx of predominately lymphocytes, granulocytes or monocytes to the site of tissue injury. Inflammation (perivascular, monocytic, granulocytic, lymphocytic and fibrosis) was independently scored 1 to 3 for mild, moderate, and severe for each type. Necrosis and hemorrhage can occur within minutes of an insult. Congestion and granulocytic inflammation could occur within 1 h, with maximum affects seen within 24-48 h post trauma. Hypertrophy, hyperplasia, lymphocytic, monocytic and perivascular inflammation can develop within 12-72 h post-trauma. A 0 score was given when lesions were not observed.

Data Analyses

For each of the eight tissue classes and every lesion type we computed the frequency (%) with which lesions occurred. Then, tissue-lesion combinations that showed abnormalities in at least 5% of the fish examined were selected for further analyses. Analyses focused on testing whether the mean scores for each tissue-lesion combination differed among electrical treatments, test species, and fish size. We also tested whether scores differed between fish that were immobilized or not immobilized within the first 3 s of the electrical treatment, between those that were tetanized or not tetanized by the end of the 15 s exposure to electricity, and between those that survived or died within the 18 h holding period after exposure. All analyses were conducted using general linear models

(Procedure GLM; SAS Institute 1996). We relaxed significance testing to $\alpha = 0.2$ because making a Type II error (i.e., accepting a null hypothesis of no effect when the alternative is true) was a major concern due to the injurious nature of the effect being tested.

Results

In all, tissues for 649 fish were analyzed, but not all tissues were available for all fish. Thus, depending on tissue type, the number of fish analyzed ranged from 532 to 631 (Table 1). The frequency with which abnormalities occurred ranged from 0 to 31%. Seven tissue-lesion combinations showed abnormalities in at least 5% of the fish examined. These included gill necrosis (14% abnormal; Table 1), gill cell change (31%), gill vascular change (5%), kidney vascular change (31%), brain vascular change (21%), heart vascular change (18%), and spleen vascular change (8%). The rest of the tissue-lesion combinations exhibited $\leq$2% abnormalities (Table 1).

Mean scores for the seven tissue-lesion combinations that showed abnormalities in at least 5% of the fish ranged from 0 to 1.56 (Table 2). Statistical analyses revealed significant effects of species, size, and electrical treatment for the scores of gill necrosis, gill cell change, kidney vascular change, brain vascular change, heart vascular change, and spleen vascular change, and only a significant species effect for gill vascular change. Various significant interactions among these variables were also evident. In general, small fish showed more lesions than large ones, but there was no consistent detectable pattern over species or electrical treatment.

Some differences were observed within the immobilization, tetany, and mortality categories. Scores of fish that were immobilized within 3 s exhibited significantly higher kidney vascular change and lower heart vascular change than those that were not (Table 3). Scores of fish that were tetanized within the 15 s treatment period showed lower spleen and heart vascular change. Scores of fish that died after electrofishing had significantly higher gill necrosis, kidney vascular changes, and brain vascular changes, and significantly lower gill cell change. However, no consistent patterns relating mortality to species, size, and electrical treatment were apparent.

Discussion

With the acute and extensive nature of insults resulting from electrofishing, the histological lesions that could result are limited. In this study, the possible changes were limited to cellular, vascular, and inflammatory changes that could occur within an 18-h period. Often, histological changes are subtle even though the overall effect on the organism may be substantial.

The greater effect on small fish may be due to the higher power levels required to immobilize these fish. Higher power densities applied through a smaller tissue area would cause more cellular damage. In the immobilized and tetanized groups, congestion was the most consistent vascular change observed; congestion alone is not a cause of death, but an indication of stress-induced changes in the circulatory system. The increased incidence of gill necrosis along with a decreased incidence of gill cell change among fish that died suggests a peracute insult. In peracute mortality, the fish die so quickly that inflammatory and cell response changes are not evident. Damage to the gill epithelial surface is more often lethal due to the critical roles of these cells in respiratory exchange, and maintenance of normal blood pH and ammonia levels. The lack of cell changes suggestive of sublethal cell insult indicates a threshold (all or none) effect of the electric treatment.

In this study, tissues from the same fish were viewed separately and were analyzed one variable at a time. Viewing was done separately with the pathologist blinded to the treatment to avoid biasing lesion detection, evaluation, and scoring. Nonetheless, histopathology may be most effective when the pathologist is allowed to take into account the lesions occurring in all tissues, to deduce the overall effect on the animal. Analysis of multiple observations on individual specimens might be better accomplished through multivariate approaches. However, multivariate approaches would have required that all tissues be available for all fish.

The variability in the initial health status of the fish also was likely a confounding influence. Most of the small and large channel catfish used in the study appeared to have previously experienced a Proliferative Gill Disease epizootic. Although effects on gill tissue can be expected when fish are treated with electricity, such effects may not be detectable when pre-existing lesions occur. In fish culture situations, it may be nearly impossible to work with specimens that do not already have lesions. While control fish were included in this study to deal with the effect of previous injuries, existing lesions may conceal the development or detection of new lesions.

Electrical treatments were associated with lesions in fish treated to various electrical settings. Nevertheless, few consistent histological changes were noted that were different from the non-electrofished controls. Histopathological examination of the controls demonstrated variability in the initial health status of the fish used in the study, and this variability may have masked subtle effects of the electric current exposure. Therefore, although legions were present in fish treated to various electrical settings, no clear trends between electrofishing and histopathology could be identified and further physiological studies are warranted.

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Table 1. Frequency of occurrence (%) of lesions in eight tissues of fish treated with six electrical waveforms described in the Methods section. Numbers in parentheses represent sample sizes.

Tissue and lesion	Control	Electrical waveform DC	110-6	110-1	15-6	15-1	CPS	All
Gills	(76)	(76)	(73)	(98)	(84)	(80)	(45)	(532)
Necrosis	13	14	15	13	4	11	29	14
Cell change	25	26	47	24	29	39	31	31
Hemorrhage	0	0	0	1	0	0	0	0
Vascular change	4	2	0	10	8	5	4	5
Inflammation	0	0	0	3	0	0	0	0
Muscle	(93)	(98)	(85)	(106)	(94)	(101)	(54)	(631)
Necrosis	0	0	0	0	0	0	2	0
Cell change	0	0	0	0	0	0	0	0
Hemorrhage	0	3	0	0	0	0	4	1
Vascular change	0	0	0	0	0	0	0	0
Inflammation	0	0	0	0	0	0	0	0
Posterior kidney	(85)	(87)	(71)	(96)	(80)	(83)	(43)	(545)
Interstitial necrosis	0	0	2	0	0	0	0	0
Interstitial cell change	0	0	2	0	0	0	0	0
Necrosis	0	0	0	0	0	0	0	0
Cell change	0	0	0	0	0	0	0	0
Hemorrhage	0	2	0	0	0	0	0	0
Vascular change	36	40	21	46	24	29	17	31
Inflammation	0	0	5	2	0	0	0	1
Liver	(92)	(97)	(82)	(109)	(91)	(101)	(54)	(626)
Necrosis	0	1	0	0	0	0	2	0
Cell change	0	0	0	0	0	0	0	0
Pancreas	(92)	(97)	(82)	(109)	(91)	(101)	(54)	(626)
Necrosis	0	0	0	0	0	0	0	0
Cell change	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Vascular change	1	3	1	7	3	0	2	2
Inflammation	0	0	0	0	0	0	0	0
Brain	(83)	(92)	(80)	(98)	(90)	(86)	(50)	(579)
Necrosis	1	0	0	0	0	1	2	1
Cell change	0	0	0	0	0	0	0	0
Hemorrhage	0	2	0	0	0	0	0	0
Vascular change	21	23	16	20	15	25	33	21
Inflammation	1	0	0	0	0	0	2	0
Heart	(83)	(90)	(72)	(103)	(83)	(82)	(50)	(563)
Necrosis	0	0	0	0	0	0	0	0
Cell change	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Vascular change	19	28	10	24	10	27	6	18
Inflammation	0	1	0	2	1	1	0	1
Spleen	(86)	(98)	(78)	(103)	(85)	(89)	(46)	(585)
Necrosis	0	0	0	0	0	0	0	0
Cell change	0	0	0	0	0	0	0	0
Hemorrhage	0	0	0	0	0	0	0	0
Vascular change	11	18	9	3	1	8	6	8
Inflammation	0	0	0	0	0	0	0	0

Table 2. Mean scores of seven tissue-lesion combinations treated with six electrical waveforms described in the Methods section. For each species, numbers in parentheses represent the mean total length (mm) followed by the mean total weight (g).

Tissue, lesion, and species	Control	Electrical waveform					
		DC	110-6	110-1	15-6	15-1	CPS
Gills necrosis	(76)	(76)	(73)	(98)	(84)	(80)	(45)
Channel catfish (162, 30)	0.00	0.25	0.00	0.00	0.00	0.00	1.00
Channel catfish (320, 283)	0.14	0.00	0.00	0.00	0.00	0.00	0.00
Bluegill (67, 5)	0.53	1.60	0.93	1.08	0.29	0.73	1.25
Bluegill (156, 80)	0.13	0.00	0.00	0.33	0.17	0.00	0.00
Largemouth bass (72, 4)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Largemouth bass (216, 135)	0.10	0.00	0.00	0.08	0.00	0.17	0.00
Gill cell change	(76)	(76)	(73)	(98)	(84)	(80)	(45)
Channel catfish (162, 30)	0.76	1.56	0.93	0.14	1.13	1.27	0.40
Channel catfish (320, 283)	0.43	0.21	0.77	0.86	0.19	0.31	0.56
Bluegill (67, 5)	0.12	0.00	0.13	0.08	0.14	0.00	0.00
Bluegill (156, 80)	0.63	0.75	0.83	0.78	0.67	0.85	0.71
Largemouth bass (72, 4)	0.13	0.13	0.54	0.00	0.00	0.23	0.00
Largemouth bass (216, 135)	0.10	0.20	0.43	0.31	0.10	0.00	0.43
Gill vascular change	(76)	(76)	(73)	(98)	(84)	(80)	(45)
Channel catfish (162, 30)	0.06	0.00	0.00	0.14	0.31	0.00	0.00
Channel catfish (320, 283)	0.07	0.71	0.38	0.10	0.19	0.08	0.11
Bluegill (67, 5)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bluegill (156, 80)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Largemouth bass (72, 4)	0.13	0.00	0.00	0.00	0.00	0.15	0.25
Largemouth bass (216, 135)	0.00	0.00	0.00	0.00	0.20	0.16	0.00
Kidney vascular change	(85)	(87)	(71)	(96)	(80)	(83)	(43)
Channel catfish (162, 30)	0.27	0.00	0.00	0.95	0.11	0.17	0.43
Channel catfish (320, 283)	0.50	0.50	0.62	0.00	0.12	0.17	0.00
Bluegill (67, 5)	0.59	0.88	0.45	0.57	0.22	0.50	0.50
Bluegill (156, 80)	0.45	0.38	0.36	0.00	0.62	0.46	0.57
Largemouth bass (72, 4)	0.07	0.40	0.27	0.25	0.14	0.25	0.00
Largemouth bass (216, 135)	0.25	0.00	0.00	0.46	0.50	0.22	0.00
Brain vascular change	(83)	(92)	(80)	(98)	(90)	(86)	(50)
Channel catfish (162, 30)	0.29	0.11	0.00	0.15	0.16	0.05	0.78
Channel catfish (320, 283)	0.23	0.19	0.38	0.27	0.06	0.31	0.40
Bluegill (67, 5)	0.31	0.22	0.36	0.36	0.27	0.23	0.44
Bluegill (156, 80)	0.10	0.18	0.14	0.00	0.07	0.08	0.33
Largemouth bass (72, 4)	0.29	0.67	0.21	0.18	0.29	0.47	0.50
Largemouth bass (216, 135)	0.00	0.00	0.00	0.07	0.20	0.36	0.00
Heart vascular change	(83)	(90)	(72)	(103)	(83)	(82)	(50)
Channel catfish (162, 30)	0.00	0.00	0.00	0.33	0.00	0.00	0.20
Channel catfish (320, 283)	0.43	0.19	0.08	0.33	0.58	0.00	0.00
Bluegill (67, 5)	0.27	0.18	0.09	0.00	0.08	0.17	0.17
Bluegill (156, 80)	0.10	0.00	0.07	0.00	0.08	0.00	0.00
Largemouth bass (72, 4)	0.25	0.93	0.29	0.42	0.80	0.33	0.00
Largemouth bass (216, 135)	0.00	0.00	0.00	0.00	0.08	0.09	0.00
Spleen vascular change	(86)	(98)	(78)	(103)	(85)	(89)	(46)
Channel catfish (162, 30)	0.07	0.50	0.00	0.00	0.00	0.00	0.30
Channel catfish (320, 283)	0.00	0.00	0.08	0.00	0.00	0.00	0.00
Bluegill (67, 5)	0.19	0.50	0.27	0.13	0.00	0.00	0.00
Bluegill (156, 80)	0.14	0.00	0.07	0.00	0.07	0.00	0.00
Largemouth bass (72, 4)	0.22	0.46	0.08	0.00	0.00	0.09	0.00
Largemouth bass (216, 135)	0.00	0.00	0.00	0.00	0.00	0.33	0.00

Table 3. Mean scores of seven tissue-lesion combinations treated with six electrical waveforms described in the Methods section. Scores are summarized according to three categories: (1) fish that were immobilized within the first 3 s of the electrical treatment; (2) fish that were tetanized during the 15 s exposure; and (3) fish that died during the 18-h holding period after exposure. The 0 and 1 subheadings represent no effect and effect, respectively. Pairs accompanied by an asterisk were significantly different (P < 0.20)

Lesion	Immobilization within 3 s		Tetany at 15 s		Mortality	
	0	1	0	1	0	1
Gills necrosis	0.22	0.21	0.20	0.23	0.20*	0.47*
Gill cell change	0.45	0.47	0.47	0.44	0.48*	0.13*
Gill vascular change	0.07	0.11	0.08	0.08	0.10	0.01
Kidney vascular change	0.24*	0.36*	0.29	0.32	0.31*	0.53*
Brain vascular change	0.28	0.23	0.26	0.22	0.23*	0.58*
Heart vascular change	0.21*	0.14*	0.20*	0.13*	0.16	0.19
Spleen vascular change	0.11	0.08	0.13*	0.06*	0.08	0.16